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GENIC ACTION AS STUDIED BY MEANS OF THE EFFECTS OF DIFFERENT DOSES AND COMBINATIONS OF ALLELES[1]

CURT STERN

University of Rochester, Rochester, New York

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INTRODUCTION

TWO types of investigation have brought us closest to problems of the immediate action of genes: studies on the effect of different dosages of a given gene, and studies on the position effect of genes. On the whole, the two types of inquiries have remained separate, but it would seem promising to concentrate both of them on one and the same gene. May not even a partial understanding of the primary action of a gene in its normal position help in interpreting its changed effect in a new position?

These considerations have led to experiments with the gene "cubitus interruptus," *ci*, located in the microchromosome, IV, of *Drosophila melanogaster*. Its favorable nature for the study of position effects was shown by DUBININ and SIDOROFF (1934); its availability for dosage studies is attested by the fact that heteroploidy for the fourth chromosome can be obtained easily. The following report deals with the effect of different doses and of combinations of the mutant allele *ci* and its wild type alleles + in their normal position. Later papers will present new data on the position effect of *ci*, and an interpretation based on the combined results of these studies.

MATERIALS AND METHODS

The stocks used primarily consisted of the Canton-Special wild type (a specially prepared stock, see below), and the following seven fourth chromosome types: cubitus interruptus (*ci*); cubitus-interruptus eyeless-Russian (*ci* ey^R); eyeless[2] (ey^2); Minute-4 (*M-4*); Minute-4[3] (*M-4*[3]); Minute-4/eyeless-dominant (*M-4*/ey^D); and haplo-IV. An extensive description of *ci* will be given later. The mutant ey^R effects a reduction in size of the compound eyes. Its expression is variable, rarely overlapping wild type and often eliminating completely one or both eyes. Although a recessive, it may be recognized phenotypically in a varying percentage of heterozygotes by a peculiar "cut-off" edge slightly limiting the size of the eye in the ventro-posterior region. The mutant ey^2 is an allele of ey^R, very similar to the latter, but of independent origin. The two Minute stocks represent deficiencies for a section of chromosome IV involving, among others, the locus of *ci* but not of *ey*. Several bands are lacking in the salivary gland chromosomes. Although of independent origin, *M-4* and *M-4*[3] appear to be indistinguishable cytologically and genetically (BRIDGES 1935a). The last mutant, ey^D, a complex chromosome aberration in the fourth chromosome which, besides other effects, produces a peculiar shape and decreased size of the compound eyes, was employed only as a "marker." Before being

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used, all stocks were made isogenic for chromosomes I, II, and III with a wild type stock derived from the "Canton-Special" wild type stock (any exception from isogeneity will be noted specifically). The Canton-Special stock had been made isogenic and its salivary gland chromosomes checked for homogeneity by C. B. BRIDGES. The derived stock employed in the present work was once more extracted as an isogenic stock from BRIDGES's stock. No isogeneity between different stocks could be obtained for the small chromosome IV due to its great rarity of crossing over. Furthermore, since the experiments extended over a period of several years, it is likely that the original isogeneity of the various stocks was disturbed by mutations. However, no striking genetic inhomogeneity was encountered.

The morphological expression of the *ci* gene consists in disturbances of the wing venation. There are slight plexus appearances, but the most characteristic effects are interruptions in the fourth or cubital vein, both proximal and distal to the posterior crossvein. All effects are variable and at the usual culture temperature of 25°–26°C overlap normal in some individuals. At lower temperatures the phenotypes are more extreme and may not overlap normal in any case. In order to measure the effect of the *ci* gene in various dosages, the flies were classified into five arbitrary classes. The frequency distributions of various constitutions could then be compared. Only the section of the fourth vein distal to the posterior crossvein was considered, and the following classes were distinguished (modifying a scheme of KHWOSTOWA 1939; see fig. 1):

class N—normal vein

class 0—a distinct thinning of the vein in one or more regions, of variable length; no complete interruption of the vein

class 1—one or more complete interruptions of the vein, the sum of the lengths of which equals less than one-half of the whole section between posterior crossvein and margin of wing

class 2—one or more complete interruptions of the vein, the sum of the lengths of which equal more than one-half of the whole section considered

class 3—complete absence of the vein between crossvein and margin of wing except, occasionally, presence of a very short section projecting beyond the crossvein, or of a dot-like distal remnant of the vein not exceeding in length the normal vein width, or of a combination of the two structures.

Both wings of each individual were inspected whenever possible, and the classification was based on both. Thus, only flies with both wings of class N were classified as such; flies in which no wing deviated more from normal than classes 0 or 1 were classified according to the more abnormal wing. In all cases of more extreme expression of *ci*, the lengths of the interrupted sections of both wings were averaged in order to place these individuals in either class 1 or 2, unless the absence of the veins in both wings determined their classification as 3. On the whole, there was a high correlation in expression of *ci*, between the two wings of an individual.

It is obvious from this description that considerable variability existed within classes 0, 1, and 2, and that the artificial subdivisions of phenotypes were in contrast to the actual continuity of variation. Consequently, to take

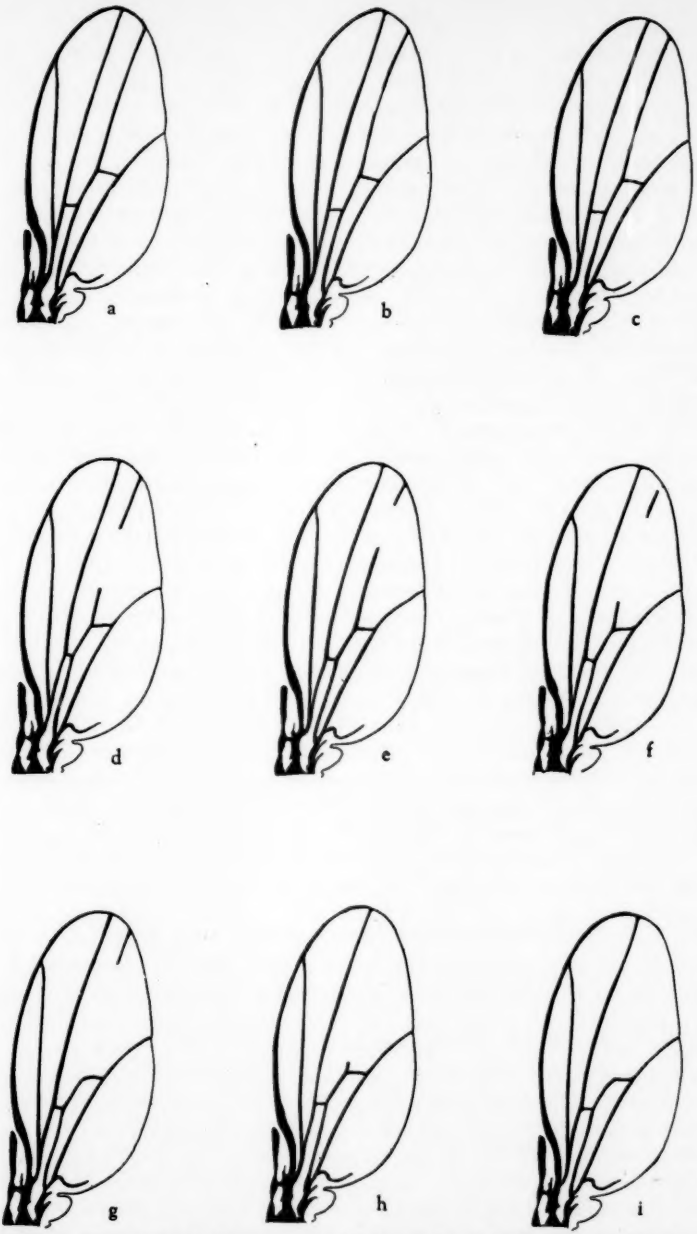

FIGURE 1. Wings of females homozygous or hemizygous for *ci*. (a) class N; (b–c) class 0; (d–e) class 1; (f–g) class 2; (h–i) class 3.

an example, flies of class 2 of a population in which also many individuals of classes 0 and 1 and none of class 3 occurred were often in the least extreme range of class 2, while flies of the same class 2 but of another population, in which many individuals belonged to class 3 and none to classes 0 and 1, tended to aggregate in the most extreme range of their class.

The fact that the variations within each of the classes distinguished were left out of consideration has probably obscured the minor differences between various populations of *ci* flies but has not interfered with the recognition of all major differences. A more accurate measurement of the wing deficiencies is possible but was not made because it was unnecessary for many comparisons, and partly because much of the evidence secured was based on progeny tests of flies classified in the described way. It would have been difficult to obtain both accurate measurements and progeny from the same large number of flies.

The use of a variable phenotype, sensitive to genetic changes, introduces the difficulty of its being also easily influenced by environmental variations. In spite of the usual attempts to provide constant culture conditions, high uniformity cannot be claimed. Apart from differences in population density, the greatest variability arises from complex changes of the food medium which at present remains uncontrolled (see GORDON and SANG 1941). However, the variability of results caused by environmental factors usually was well below that due to the different genetic constitutions tested. Furthermore, in nearly all cases the experiments were so arranged that different genetic constitutions occurred among the offspring of the same parents and developed under conditions, as identical, as found within the same bottle. Under these circumstances duplicable results were obtained.

Three different temperatures were used, which will be referred to as 26°, 18°, and 13°. The 26° incubator varied only by a small fraction of a degree. The lower temperatures could not be kept constant within narrow limits during periods of excessive summer heat. The 18° incubator rarely went as low as 17° but occasionally as high as 20° or even higher, while the 13° incubator fluctuated between 12° or lower and 15.5°. It will be seen later that these irregularities did not influence the results within the scope of this work.

THE EFFECT OF ONE TO THREE DOSES OF CI (AT 26°)

There are two methods of changing the dosage of a given allele. One involves the substitution of various numbers of one allele for another allele, as in the well known case of corn endosperm, where from one to three dominant yellow alleles may be introduced into this triploid tissue in place of the recessive white alleles at the Y locus. In this method no direct comparison of the phenotypic effect of dosage differences of the allele in question can be made since the phenotypes depend on all three alleles together, rather than on the dosage differences of only one allele, as, for example, three yellows plus no white vs. two yellows plus one white vs. one yellow plus two whites, etc. The other method of obtaining dosage differences avoids this difficulty. It makes use of deficiencies and duplications and thus builds up different multiples of one allele without involving heterozygous conditions. Among the studies in which

this method was employed there are those on deficiencies (MOHR 1923), on the dosage effects of the Y chromosome-borne gene bobbed (STERN 1929), on duplications of sections of the X chromosome (MULLER, LEAGUE, and OFFERMANN 1931), and on dosage effects of the fourth chromosome gene "shaven" in flies haploid to triploid for this chromosome (SCHULTZ 1935). An apparent disadvantage of this more direct method lies in the fact that rarely is it only the gene under consideration which is cumulated. In most cases, loci adjacent to the test gene are also involved. As a matter of fact, phenotypic differences between flies with various doses of a given allele were originally thought to be due to neighboring genes in the whole duplicated or deficient sections. This view followed from the theory of genic balance which expects "a character change whenever the ratio of plus to minus modifiers within the section is different, for any character, from that of the whole normal group" (MORGAN, BRIDGES, and STURTEVANT 1925, p. 167). Later, GOLDSCHMIDT (1927) gave reasons for neglecting the adjacent genes, a view which became generally accepted. For example, in SCHULTZ'S studies of shaven, all data were interpreted in terms of the dosage differences of shaven itself, without reference to the nine other known loci in the fourth chromosome involved and the presumably still larger number of unknown loci. Still more recently, GOLDSCHMIDT (1938) has returned to the consideration of larger chromosome units than a single locus.

In the present study of the fourth chromosome gene *ci*, an attempt was made to alter as little of the fourth chromosome as possible, except the *ci* locus, by making use of the deficiency *M-4*. This deficiency is large enough to include four out of the seven known loci tested, but the actual fraction of the fourth chromosome lost, as seen cytologically in salivary gland chromosomes, is very much smaller than that suggested by the number of known loci affected. The *M-4* chromosome possesses presumably a normal left arm with its approximately 12 bands (GRIFFEN and STONE 1940) and a right arm which retains about 25 of the 29 bands labelled by BRIDGES (1935a). The care in keeping the major part of chromosome IV as unaltered as possible proved to be justified.

Under these conditions several comparisons were available: between the effects of one vs. two *ci* alleles in the diplo-IV constitutions *ci/M-4* and *ci/ci*, and in the triplo-IV flies *ci/M-4/M-4* and *ci/ci/M-4*, and between the effects of two vs. three *ci* alleles in triplo-IV *ci/ci/M-4* and *ci/ci/ci* flies.

THE EFFECT OF ONE VS. TWO CI ALLELES

Table 1 summarizes results from the cross of *ci* ey^R/*M-4* by *ci* ey^R/*ci* ey^R which yields offspring of the two parental types easily distinguishable by the normal eyes and Minute-bristles of one genotype and the *ey* type eyes and normal bristles of the other. It is seen that the males were more extreme *ci* than the females. This relation was met with again in all other experiments. Within each sex the phenotypes of flies with a single dose of *ci* deviate more from normality than those of flies with two doses of *ci*, the differences being fully significant. A similar result had been obtained by MOHR (1933). How-

ever, before accepting these findings as due to dosage effects, we must inquire as to other possible sources of the observed variations in phenotype.

TABLE 1

F_1 of ci ey^R/ci ey^R×ci ey^R/M-4

(In this and other tables the results include both reciprocal crosses unless indicated otherwise.)

	ci ey^R/ci ey^R					*ci ey^R/M-4*				
	N	0	1	2	3	N	0	1	2	3
♀♀	14	46	149	74	1	—	1	16	122	41
♂♂	5	16	112	150	19	—	—	2	108	52

	χ^2*	n	P
♀♀	201	4	<0.01
♂♂	118	3	<0.01

* For these and all other calculations of χ^2 classes were combined whenever the expected numbers fell below five. In cases of twofold tables in which the expected numbers were below five Yates' correction was applied.

Part of the variability evident in the various distributions can be shown to be correlated with an external condition, the hatching order. Early hatching individuals are often less extreme *ci* than later ones of the same constitutions. This is shown in table 2 where flies hatched during approximately the first

TABLE 2

Early versus late hatching.

F_1 of ci ey^R/ci ey^R×ci ey^R/M-4

(These are the same flies as recorded in table 1 except for one culture omitted here in which nearly all individuals hatched during the early hatching period.)

	HATCHED DURING	*ci ey^R/ci ey^R*					*ci ey^R/M-4*				
		N	0	1	2	3	N	0	1	2	3
♀♀	10th–14th day	14	32	83	30	—	—	—	3	40	12
	15th–20th day	—	13	57	43	1	—	1	14	76	27
♂♂	10th–14th day	5	12	70	67	5	—	—	—	28	9
	15th–20th day	—	2	39	81	14	—	—	2	77	41

	♀♀			♂♂		
	χ^2	n	P	χ^2	n	P
ci ey^R/ci ey^R	22	2	<0.01	24	3	<0.01
ci ey^R/M-4	2.28	2	0.32	1.26	1	0.26

and second half of an 11-day hatching period are compared. Among the *ci ey^R* flies of both sexes a shift toward more extreme interruption of the cubital vein

in the later hatches is evident and statistically significant, though no striking shift had occurred among the Minute flies. In other experiments, however, similar shifts were observed among Minute flies (table 3). In view of the fact

TABLE 3

Early versus late hatching.

ci ey^R/M-4 from eggs layed during a two-day period.

		N	0	1	2	3
♀♀	early hatch	—	6	17	39	4
	late hatch	—	1	23	78	26
♂♂	early hatch	—	1	11	40	—
	late hatch	1	1	8	86	33

	χ^2	n	P
♀♀	10.4	2	<0.01
♂♂	21	2	<0.01

that the Minute flies typically hatch from one to several days later than the non-Minutes, it is necessary to investigate whether this shift in expression might be strong enough to be responsible for the differences ascribed to the dosage effect of *ci*. The data of table 2 do not support such an assumption, since even the later hatching *ci ey^R/ci ey^R* flies are far more normal, as a group, than the early *ci ey^R/M-4* flies. A still better test was made in the following experiment. Instead of permitting flies to lay eggs in a culture for eight to ten days, as usual, parents like those in table 1 were left for 24 hours in culture

TABLE 4

Early versus late hatching.

F$_1$ of ci ey^R/ci ey^R×ci ey^R/M-4

From cultures in which eggs were deposited for 24 hours (in some 26 hours). The *ci ey^R/ci ey^R* individuals hatched from 24 to 48 hours later (in some from 26 to 52 hours later) than the *ci ey^R/M-4* flies.

		N	0	1	2	3
♀♀	*ci ey^R/ci ey^R*	216	137	243	16	1
	ci ey^R/M-4	60	79	293	176	2
♂♂	*ci ey^R/ci ey^R*	95	84	269	31	—
	ci ey^R/M-4	18	30	232	211	—

	χ^2	n	P
♀♀	241	3	<0.01
♂♂	215	3	<0.01

bottles (in one set of cultures for 26 hours) and then removed. In order to test the influence of hatching order, the offspring were collected at 24- (or 26-) hour intervals and the Minute flies of any hatching period compared with the non-Minutes of the second hatching period following that of the Minute flies. Since the length of the collecting periods was identical with that of the parental egg laying period, the non-Minute flies used for the comparisons underwent, as a minimum, the same length of developmental period as the Minute flies and generally even surpassed them in developmental duration. Table 4 shows that in spite of this the earlier observed effect of the dosage difference of *ci* persists and is neither obliterated nor simulated by the shift due to hatching order.

Another possible source of the differences between flies with one and two doses of *ci* lies in the fact that the two types are phenotypically different in regard to ey^R and *M-4*. It was conceivable that these conditions exert a modifying effect on the expression of *ci* so that ey^R non-Minute flies are less extreme *ci* than not-*ey* Minute, regardless of dosage differences of *ci*. This possibility could easily be tested for ey^R by raising offspring of a cross from which both *ey* and normal-eyed homozygous *ci* flies result. It could not be tested as directly for *M-4*, since the presence of *M-4* is indissolubly bound up with the hemizygous condition of *ci* under consideration. Since, however, the phenotypic effects of Minute loci in chromosomes other than the fourth are very similar to each other and to that of *M-4*, it was decided to substitute another Minute, the third chromosome *M(3)-w*, for *M-4*. By crossing flies of the constitution *M(3)-w*/+; *ci* ey^R/*ci* with *ci* ey^R/*ci* ey^R, the effects of *M(3)-w* and *ey* were tested both separately and together (table 5; the *M(3)-w* parents were not isogenic with the rest of experiments). The most important comparison is that of *ci* ey^R/*ci* ey^R with *M(3)-w*/+; *ci* ey^R/*ci* (table 5, comparison 1) in which the phenotypes are *ci* ey^R and *ci M* as in the dosage experiment. The difference between the distributions in respect to the *ci* classes is significant in both sexes without, however, indicating a shift similar to that observed with the dosage data. Instead, in the females the trend is slightly in the opposite direction, while in the males the distributions differ in dispersion as seen in table 5 so that the largest contributions to χ^2 are derived from classes on both ends of the distribution. Such differences may well be due to genetic inhomogeneity introduced by the *M(3)-w* stock. Thus the results with both females and males indicate that the phenotypic difference in regard to *ey* and *M* in *ey*, not-Minute vs. not-*ey*, Minute is not responsible for the observed shift of phenotypes in the *ci* dosage experiment. The data in table 5 also permit comparisons, in each sex, between flies with or without *M(3)-w* but alike otherwise [(2) and (3)]. Three of the four comparisons differ significantly but not in any common direction. Thus, *M(3)-w* seems not to be a modifying factor for *ci*. Further comparisons, between flies with or without *ey* phenotype, but alike otherwise [(4) and (5)], yielded no significant differences in three cases, while the fourth comparison showed a shift toward normality among not-*ey* flies. Finally, comparisons between flies with or without both *M(3)-w* and *ey* phenotypes [(6)]

TABLE 5

F_1 of $M(3)$-w/+; ci ey^R/ci×ci ey^R/ci ey^R.

	♀♀					♂♂				
	N	0	1	2	3	N	0	1	2	3
ci ey^R/ci ey^R	—	27	110	158	8	—	6	57	241	17
ci ey^R/ci	3	30	127	185	15	—	8	64	279	42
$M(3)$-w/+; ci ey^R/ci ey^R	—	5	37	58	7	—	5	18	104	24
$M(3)$-w/+; ci ey^R/ci	—	30	60	78	5	—	16	29	124	27

	♀♀			♂♂		
	χ^2	n	P	χ^2	n	P
(1) *ci ey^R/ci ey^R* vs. *$M(3)$-w/+; ci ey^R/ci*	7.58	2	0.02	24	3	<0.01
(2) *ci ey^R/ci ey^R* vs. *$M(3)$-w/+; ci ey^R/ci ey^R*	2.38	2	0.3	15	2	<0.01
(3) *ci ey^R/ci* vs. *$M(3)$-w/+; ci ey^R/ci*	8.05	3	0.05	14	3	<0.01
(4) *ci ey^R/ci ey^R* vs. *ci ey^R/ci*	1.18	3	0.8	6.86	3	0.08
(5) *$M(3)$-w/+; ci ey^R/ci ey^R* vs. *$M(3)$-w/+; ci ey^R/ci*	10.5	2	<0.01	4.51	3	0.2
(6) *$M(3)$-w/+; ci ey^R/ci ey^R* vs. *ci ey^R/ci*	3.17	3	0.4	3.10	2	0.2

gave no significant differences. Thus, neither *$M(3)$-w* nor *ey* separately or combined seem to be modifiers of *ci*.

In view of the failure to discover either environmental or secondary genetic factors as responsible for the typical differences between *ci* and *ci/ci* flies, the phenotypic effect observed is considered a true dosage effect.

In addition to the comparison of the effects of one and two doses of *ci* in diplo-IV flies, some data relating to the same dosage difference are available

TABLE 6

F_1 of ci ey^R/ci ey^R/M-4 ♀♀ ×ci ey^R/M-4 ♂♂
(Records for *ci ey^R* phenotypes omitted.)

	♀♀					♂♂				
	N	0	1	2	3	N	0	1	2	3
ci ey^R/ci ey^R/M-4	39	33	46	21	1	7	22	54	32	1
ci ey^R/M-4 plus *ci ey^R/M-4/M-4*	4	7	28	39	6	1	3	28	85	7
of the above, certainly *ci ey^R/M-4/M-4* (cf. table 8)	—	2	6	2	—	—	—	3	2	—

ci ey^R/ci ey^R/M-4 ♀♀ vs. *ci ey^R/M-4/M-4 ♀♀*: $\chi^2 = 2.53$; n = 1; P = 0.1.

from triplo-IV flies *ci ey^R/M-4/M-4* vs. *ci ey^R/ci ey^R/M-4*. The phenotypic difference between wild type flies with two or three microchromosomes is so slight that it is impossible to separate them visually with any degree of certainty. It is therefore necessary to make progeny tests unless the presence of mutant marker genes in some of the fourth chromosomes produces a phenotypic distinction. Triplo-IV flies with one and two doses of *ci* were obtained from a cross of triplo-IV *ci ey^R/ci ey^R/M-4* females and *ci ey^R/M-4* males (table 6). All non-eyeless, non-Minute-bristled flies are *ci ey^R/ci ey^R/M-4*, while the non-eyeless Minute flies are expected to be a mixture of *ci ey^R/M-4* and *ci ey^R/M-4/M-4*. By breeding the Minute flies individually to *ci ey^R/ci ey^R* mates, they could be assigned to one or the other of the possible constitutions. As will be shown later in detail, a very high amount of sterility was encountered, and only 15 individuals were proven definitely to have been *ci ey^R/M-4 /M-4*. They are entered in the last horizontal row of table 6. Though the numbers are too low to be significant, their distributions are in line with the earlier conclusion that two doses of *ci* produce a more normal vein phenotype than one dose.

THE EFFECT OF TWO VS. THREE CI ALLELES

The final comparison made was between the effects of two and three *ci* doses in triplo-IV individuals of the constitutions *ci ey^R/ci ey^R/M-4* and *ci ey^R/ci ey^R/ci ey^R*. Such flies were obtained in the offspring of *ci ey^R/ci ey^R /M-4* and *ci ey^R/ci ey^R* parents. While the *ci ey^R/ci ey^R/M-4* F_1 was recognizable by its non-*ey*, normal bristled phenotype, the *ci ey^R/ci ey^R/ci ey^R* offspring were not clearly distinguishable phenotypically from their *ci ey^R/ci ey^R* diplo-IV sibs. Progeny tests had to be employed. In most experiments, they were restricted arbitrarily to females. Since the two sexes have different phenotypic distributions over the *ci* range, the limitation to one sex permitted the accumulation of more extensive data within homogeneous material. By recording the *ci* phenotype of each F_1 *ci ey^R* female and then mating her as a virgin either to several *ci ey^R/M-4* males or to *ey^D/M-4* males, it was possible to ascertain whether the female was diplo- or triplo-IV. In the former case all normal eyed F_2 flies were Minute, in the latter case many of them were normal bristled. The results of these tests are summarized in table 7.

In the progeny experiments a considerable number of the phenotypically *ci ey^R* females to be tested remained without offspring. It was of importance to ascertain whether this sterility occurred more often in the diplo-IV than in the triplo-IV flies or *vice versa*, since a selective sterility would have affected the significance of the distributions of the fertile diplo-IV and triplo-IV females. Since tests for homogeneity between the distributions of the sterile flies on the one side and the sum of the fertile diplo-IV and triplo-IV *ci ey^R* types on the other side in no case yielded significant differences, it may be assumed that the sterility affected diplo-IV and triplo-IV flies indiscriminately. Independent evidence for this conclusion is seen in experiment (b) where the unusually high frequency of sterile test cultures was apparently due to the males employed, and not to the females tested.

It was now possible to proceed to the essential comparison that of *ci ey^R /ci ey^R/M-4* and *ci ey^R/ci ey^R/ci ey^R* flies. The distribution of triplo-IV flies with two doses of *ci* was shifted in all cases toward a more extreme phenotype than that of flies with three doses. The difference is only at the borderline of significance in the small experiment (a), but highly significant in the large experiments (b) and (c). Further data permitting comparisons between triplo-

TABLE 7

F_1 of ci ey^R/ci ey^R/M-4 × ci ey^R/ci ey^R
(Records for *ci ey^R/M-4* omitted.)

EXPERIMENT	SEX	GENOTYPE	N	0	1	2	3
(a)	♀♀	*ci ey^R/ci ey^R/M-4*	—	5	18	19	—
		ci ey^R/ci ey^R/ci ey^R	—	3	1	1	—
		ci ey^R/ci ey^R	—	—	4	8	—
		sterile	—	—	1	1	—
(a)	♂♂	*ci ey^R/ci ey^R/M-4*	2	6	26	26	—
		ci ey^R/ci ey^R/ci ey^R	—	—	3	—	—
		ci ey^R/ci ey^R	—	—	6	8	1
		sterile	—	—	1	3	—
(b)	♀♀	*ci ey^R/ci ey^R/M-4*	3	75	144	102	6
		ci ey^R/ci ey^R/ci ey^R	—	23	19	9	—
		ci ey^R/ci ey^R	—	10	55	49	5
		sterile	1	15	40	24	4
(c)	♀♀	*ci ey^R/ci ey^R/M-4*	14	41	148	73	1
		ci ey^R/ci ey^R/ci ey^R	11	36	39	12	—
		ci ey^R/ci ey^R	4	9	58	66	3
		sterile	3	6	8	7	—

		χ^2	n	P
ci ey^R/ci ey^R/M-4 vs. *ci ey^R/ci ey^R/ci ey^R*	((a) ♀♀)	4.32	1	0.04
	(b)	11	2	<0.01
	(c)	30	3	<0.01
ci ey^R/ci ey^R/ci ey^R vs. *ci ey^R/ci ey^R*	((a) ♀♀)	5.12	1	0.02
	(b)	33	2	<0.01
	(c)	58	3	<0.01
ci ey^R/ci ey^R/M-4 vs. *ci ey^R/ci ey^R*	((a) ♀♀)	1.71	1	0.2
	((a) ♂♂)	1.33	1	0.2
	(b)	14	2	<0.01
	(c)	23	3	<0.01
ci ey^R/ci ey^R plus *ci ey^R/ci ey^R/ci ey^R* vs. sterile	(b)	0.43	2	0.8
	(c)	1.70	2	0.4

IV flies with two and three doses of *ci* were obtained in experiments primarily designed for other purposes (see tables 10, 11, 13, and 19). In general, they are

in conformity with the present results. Thus, a cumulative dosage effect of three versus two *ci* alleles is established.

THE EFFECT OF THE RESIDUE OF CHROMOSOME IV (AT 26°)

Were the considerations justified which led to the use of the deficient *M-4* chromosome instead of simpler comparisons of flies with multiples of whole fourth chromosomes containing *ci*? Or are the dosage effects exclusively dependent on the *ci* locus by itself? These questions as to the effect of the "residue" of the fourth chromosome on the expression of *ci* can be answered by data mainly provided by the experiments just reported.

Comparisons are based on flies in which the number of *ci* alleles is held constant while the number of fourth chromosomes deficient for *ci* is variable. In one group of genotypes only one *ci* allele was present, while the number of fourth chromosome residues varied from none to two: *ci*/o (=haplo-IV),

TABLE 8

Comparison of flies containing one dose of ci plus variable doses of the M-4 or M-4³ chromosome. Rows 3–5 all derived from a single experiment, all other rows from different individual experiments.

	♀♀					♂♂				
	N	o	1	2	3	N	o	1	2	3
ci/o	—	—	—	1	3	—	—	—	1	9
ci/*M-4*	1	8	15	18	1	—	5	17	38	7
ci ey^R/*M-4*	—	2	12	21	2	—	—	5	25	3
ci ey^R/*M-4*/*M-4*	—	2	6	2	—	—	—	3	2	—
sterile or doubtful*	4	3	9	14	4	1	3	19	57	3
ci ey^R/*M-4*³	—	—	4	20	5	1	—	7	20	5

	♀♀			♂♂		
	χ^2	n	P	χ^2	n	P
ci ey^R/*M-4* vs. *ci ey*^{R}/*M-4*/*M-4*	4.06	1	0.04	2.92	1	0.09
ci ey^R/*M-4* vs. "sterile, etc."	2.45	1	0.1	2.04	1	0.2
ci ey^R/*M-4*/*M-4* vs. "sterile, etc."	2.18	1	0.1	1.06	1	0.3

* See text.

ci/*M-4* or *ci ey*^{R}/*M-4*, and *ci ey*^{R}/*M-4*/*M-4*. In another group of genotypes two *ci* alleles were present, while the fourth chromosome residue varied from none to one: *ci ey*^{R}/*ci ey*^{R} or *ci ey*^{R}/*ci*, and *ci ey*^{R}/*ci ey*^{R}/*M-4* or *ci ey*^{R}/*ci*/*M-4*.

Only a few individuals are available for the group of genotypes containing one *ci* allele (table 8, rows 1–5). Some definite conclusions, however, may be drawn from them. In order to obtain haplo-IV flies possessing *ci* in their single fourth chromosome, a cross was made between an isogenic wild haplo-IV stock and the *ci* stock. The reason for not using the *ci ey*^{R} stock was due to BRIDGES'S (1921) statement that about 98.7 percent of haplo-IV *ey* flies die during the pupal stage. The cross with *ci*, however, was no more successful. Though equality between the diplo-IV and haplo-IV offspring was theoretically expected, about 10,000 of the former (from 42 cultures) were accompanied

by only 14 haplo-IV flies. Hundreds of other haplo-IV individuals were incapable of hatching from their pupal cases, so that a determination of their *ci* phenotype was not feasible. Fortunately the 14 hatched individuals gave a clear result. No fewer than 12 of them belong to the most extreme phenotypic class 3, the others to the still extreme phenotypic class 2. The distributions for both sexes are more extreme than any ever met with in *ci ey*^{R}/*M-4* or in *ci*/*M-4* flies (tables 1, 8, and other unpublished observations). The striking result is thus apparent that the addition of an *M-4* chromosome to a single *ci* allele leads to a more normal phenotype. In other words, the main body of the fourth chromosome, exclusive of the *ci*-including region which is absent in *M-4*, has an effect identical in direction to that of the *ci* gene itself.

The second comparison between flies with one and two *M-4* chromosomes besides a *ci ey*^{R} chromosome was attempted by mating *ci ey*^{R}/*ci ey*^{R}/*M-4* females to *ci ey*^{R}/*M-4* males and then separating, by means of progeny tests, the diplo- and triplo-IV flies among the Minute type offspring. The Minute F_1 flies were mated singly to *ci ey*^{R} flies. The next generation, by either lacking or possessing normal appearing *ci ey*^{R}/*ci ey*^{R}/*M-4* flies, indicated the genotype of the unknown parent.

A very high number, 114 out of 202, of completely sterile tests occurred, a characteristic of phenotypically *ci ey*^{R}/*M-4* which here obviously could not be overcome by the use of several individuals. To these undetermined must be added three individuals whose constitution as diplo-IV could not be definitely established. It is probable that the sterile or doubtful groups do not represent a random sample of the total. The data, however, are not large enough to be significant, particularly since the necessary combining of classes with few individuals serves to decrease excessively the contributions to χ^2. There is a trend, among the females and males, toward greater normality among the undetermined (sterile or doubtful) group than among the *ci ey*^{R}/*M-4* flies. Furthermore, the recognized *ci ey*^{R}/*M-4*/*M-4* individuals number only about one-quarter of the *ci ey*^{R}/*M-4* individuals, though on a chance basis they are expected to have a frequency of two-thirds of the latter. Very likely the undetermined group contains a disproportionately large share of *ci ey*^{R}/*M-4* /*M-4* flies. In spite of its small size the group of ten recognized *ci ey*^{R}/*M-4*/*M-4* females is significantly more normal than the group of *ci ey*^{R}/*M-4* females, and even the five males of the former constitution are distributed more toward the normal phenotype than the males of the latter constitution. These facts, together with the tendency of the undetermined flies to be more normal than the *ci ey*^{R}/*M-4* flies, make it highly probable that the addition of two doses of the "residue" of the fourth chromosome to a fourth chromosome containing *ci* is more powerful in tending toward a normal phenotype than one such dose, thus extending the conclusion from the preceding experiment.

The third type of comparison, that of flies with two *ci* fourth chromosomes, with and without an additional *M-4* chromosome, is available from experiments discussed earlier. Table 7, rows 1 and 3 of each experiment, shows that the distributions of *ci ey*^{R}/*ci ey*^{R}/*M-4* flies are shifted toward a more normal expression of *ci* than those of *ci ey*^{R}/*ci ey*^{R} flies, the shift being statistically

significant in the two large experiments (b) and (c). As in the preceding set of comparisons, the main body of the fourth chromosome has an effect identical in direction to that of the *ci* allele itself.

The validity of this result was confirmed by an additional experiment devised in order to test a possible objection. This concerns the phenotypic difference between the *ci ey^R/ci ey^R* flies (all eyeless) and the *ci ey^R/ci ey^R/M-4* flies (all normal-eyed). Although the data given in table 5 had not shown any distributional effect due to normal-eyed phenotype comparable to the distributional shift in the normal-eyed flies in table 7, the results regarding the action of the fourth chromosome residue *M-4* flies seemed important enough to be checked. This was done by an experiment in which one of the *ci ey^R* chromosomes was replaced with a *ci* chromosome. The parental cross in this case was between *ci ey^R/ci ey^R/M-4* and *ci/ci* flies. Here the offspring consist of *ci ey^R/ci*, *ci ey^R/ci ey^R/ci*, and *ci ey^R/ci/M-4* which are all normal-eyed and thus phenotypically not distinguishable. A complete separation of genotypes was accomplished in two successive generations. In the first, more than 300 virgin females assigned to the various *ci*-classes, were mated individually to *ci ey^R/ci ey^R* males. In 103 of these progeny tests *ci ey^R/M-4* F_1 flies appeared, thus marking their P-females as of the constitution *ci ey^R/ci/M-4*. The remaining 190 fertile cultures were derived from both *ci ey^R/ci* and *ci ey^R/ci ey^R /ci* parents. The F_1 eyeless flies of the former parents should have been all diplo-IV, while the F_1 eyeless flies of the latter parents should include both diplo-IV and triplo-IV individuals. On a random basis two diplo-IV to one triplo-IV would be expected in the F_1; however, the frequency of these two types could not be predicted accurately in view of the unknown extent of preferential segregation in the mother (see STURTEVANT 1936). The existence or non-existence of triplo-IV flies among the F_1 which marked their mothers as triplo-IV or diplo-IV was determined by a second generation progeny test. From ten to 12 virgin eyeless females (only rarely fewer) of the most normal *ci* type were selected. Among these the proportion of triplo-IV flies, if present at all among the F_1, should be high, since it had been shown that the triplo-IV flies are characterized by the greatest approach toward a normal phenotype. The ten to 12 F_1 flies were subdivided into three about equal groups and each group mated to several *ey^D/M-4* males. The offspring of each group was then inspected for the occurrence of the normal-eyed, not-Minute flies which result from the constitution *ci ey^R/ci ey^R/M-4*. Such flies hatched in from one to three cultures of 119 groups; they were absent in the cultures of 73 groups. The 119 grandmothers of the former groups were thus recognized as triplo-IV, the 73 of the latter groups as diplo-IV.

Before coming to the essential point of the experiment and referring to the distribution of *ci* phenotypes within these two types, two possible sources of error should be pointed out in assigning the flies to the diplo-IV or triplo-IV constitution. On the one hand it is to be expected that some parental triplo-IV flies did not happen to be represented by fertile triplo-IV offspring among the ten to 12 females selected. In this case no triplo-IV flies would appear in any of three cultures in the second progeny test, and the original parent would

therefore be wrongly classified as diplo-IV. An estimate of the error committed may be attempted on the basis of the number of cases in which all three progeny cultures, only two of them, or only one of them, produced triplo-IV offspring. These numbers, together with the distribution of the grandparents of the triplo-IV offspring, are given in table 9, which includes only those groups in which all three cultures were fertile. There were observed 16 sets of three cultures, all of which contained triplo-IV offspring, 34 sets with two cultures of this type, and 44 with one culture. On the basis of these data, an estimate, which makes χ^2 minimal, of the probability of finding a culture with triplo-IV offspring yields a value very close to 0.5. Accordingly the number of sets of three cultures in which no single culture happened to produce triplo-IV flies

TABLE 9

Frequency of sets of three cultures of which from one to three contained triplo-IV progeny.

NUMBER OF TRIPLO-IV CONTAINING CULTURES IN A SET OF THREE	PHENOTYPE OF GRANDMOTHER			TOTAL NUMBER OF SETS OF CULTURES
	0	1	2	
3	7	7	2	16
2	16	12	6	34
1	20	18	6	44

is regarded as about equal to the number with all three cultures showing triplo-IV flies. This number is 16, or less than 14 percent of the grandparental females classified as triplo-IV and about 22 percent of the grandparental females classified as diplo-IV. Counteracting this source of error is the other: In a portion of the final experiment, due probably to some unusual culture conditions, difficulties were encountered in distinguishing abnormally large "eyeless" eyes from normal ones. If mistakes were made, they would have resulted in regarding diplo-IV cultures as triplo-IV. (Even if errors occurred, they could hardly have been responsible for the strange ratio of diplo-IV to triplo-IV flies among the grandparental females as established by these tests. This ratio is 73:117 instead of the chance expectation of 2:1. No cause can be assigned to this discrepancy unless it be excessive preferential segregation. Similarly, great deviations in the observed ratio of diplo-IV to triplo-IV flies appeared in various other experiments, summarized in tables 7, 8, 11, and 13.)

What bearing have the possible errors in classification on our problem? Since it will be shown that the phenotypic distributions of diplo-IV and triplo-IV *ci* individuals in this experiment, as in the earlier ones, differ, and that the triplo-IV distribution is shifted more and the diplo-IV less toward the normal end than the distribution of *ci ey*^{R}*/ci/M-4*, it is obvious that any error in assigning parental females to one of the two classes will tend to decrease actual differences. Thus, observed differences will give minimum estimates.

We may now consider the result of the experiment devised to compare *ci ey*^{R}*/ci/M-4* and *ci ey*^{R}*/ci* flies in its bearing on the question of the influence of the residue of the fourth chromosome on the *ci* phenotype (table 10). The

first three rows of this table are of primary importance. They, as well as the last row, are self-explanatory. The flies in the row designed "doubtful" consisted of fertile, phenotypically *ci ey*^{R} females among whose grandchildren were found from only one to three triplo-IV flies. This number is so small that it is questionable whether the grandmother in each of these cases was triplo-IV herself. She may rather have been diplo-IV, and the presence of triplo-IV grandchildren might be due to new occurrences of this condition which, in our experiments, have been unexpectedly frequent. The interpretation of all or most "doubtful" females as belonging among the *ci ey*^{R}/*ci* class fits well into the distribution of the latter group. They were excluded, however, from com-

TABLE 10

F_1 ♀♀ *of ci ey*^{R}/*ci ey*^{R}/*M-4* ♀♀ × *ci*/*ci* ♂♂.
(Records of *ci*/*M-4* omitted.)

	N	O	I	2	3
ci ey^{R}/*ci*/*M-4*	1	30	53	19	—
ci ey^{R}/*ci ey*^{R}/*ci*	2	53	46	18	—
ci ey^{R}/*ci*	—	12	37	20	4
doubtful*	—	5	11	6	—
sterile	—	4	7	—	—

* See text.
ci ey^{R}/*ci*/*M-4* vs. *ci ey*^{R}/*ci*: χ^2=6.90; n=2; P=0.03

parisons of the essential distributions, those of the types *ci ey*^{R}/*ci*/*M-4* versus *ci ey*^{R}/*ci*. It is apparent that even under the conditions of this experiment, where an influence of the difference in eye size was excluded, there is clearly a contribution by the fourth chromosome outside the *ci* region toward normality of the vein.

THE EFFECT OF THE M-4^{3} CHROMOSOME (AT 26°)

The preceding experiment establishes the effect of the residue of the fourth chromosome as represented in the *M-4* chromosome. It seemed desirable in addition to test fourth chromosomes of other origins in order to ascertain whether the observed effect was attributable to fourth chromosomes in general. To have had available a series of fourth chromosomes, preferably with deficiencies of various lengths, would have been ideal. Unfortunately, only one other fourth chromosome deficiency besides *M-4* is known—namely, *M-4*^{3}, and it has been stated to be genetically and cytologically indistinguishable from *M-4*, though of independent origin (BRIDGES 1935b).

Two sets of data are available for the study of the effect of *M-4*^{3} on the *ci* phenotype. The first is the comparison between *ci*/o (haplo-IV) genotype listed in table 8, row 1, and *ci ey*^{R}/*M-4*^{3} flies, row 6. Clearly, the "addition" of the *M-4*^{3} chromosome has shifted the phenotypic distribution of *ci* toward normality. The residue of the fourth chromosome represented by *M-4*^{3} has an effect on the *ci* phenotype of the same kind as *M-4*. A direct comparison be-

tween the action of *M-4*^{3} and *M-4* has not been made. No very striking difference between *ci ey*^{R}/*M-4*^{3} and *ci ey*^{R}/*M-4* flies is apparent, but a decision could be reached only if it were possible to rear both types together in the same cultures and still distinguish them.

The second set of comparisons between flies with and without the *M-4*^{3} chromosome was made between *ci ey*^{R}/*ci ey*^{R} and *ci ey*^{R}/*ci ey*^{R}/*M-4*^{3} females. The technique involved progeny tests as in former experiments. A preliminary experiment, carried out on a small scale with stocks not fully isogenic did not give clear results (table 11(a), rows 1 and 3). A second larger and isogenic experiment (table 11(b), rows 1 and 3) was more decisive. Here the normal

TABLE 11

F$_1$ ♀ ♀ *of ci ey*^{R}/*ci ey*^{R}/*M-4*^{3}×*ci ey*^{R}/*ci ey*^{R}.
(Records of *ci ey*^{R}/*M-4*^{3} omitted.)

	N	0	1	2	3
(a) *ci ey*^{R}/*ci ey*^{R}/*M-4*^{3}	5	15	23	12	—
ci ey^R/*ci ey*^{R}/*ci ey*^{R}	8	8	10	3	—
ci ey^R/*ci ey*^{R}	4	6	19	7	1
(b) *ci ey*^{R}/*ci ey*^{R}/*M-4*^{3}	5	17	83	80	—
ci ey^R/*ci ey*^{R}/*ci ey*^{R}	6	9	27	6	—
ci ey^R/*ci ey*^{R}	—	5	48	58	3
sterile*	1	1	7	—	—

ci ey^R/*ci ey*^{R}/*M-4*^{3} vs. *ci ey*^{R}/*ci ey*^{R}	χ^2	n	P
(a)	1.03	2	0.3
(b)	6.09	2	0.05

* Only one progeny test proved sterile in experiment (a).

class was represented only among the *M-4*^{3} flies, while the most extreme class 3 was found solely among the diplo-IV flies. The modal class in the former group was class 1, while in the latter it was class 2. A χ^2 test with classes N and 0 and classes 2 and 3 combined gives a probability of somewhat less than 0.05 for the difference in distribution as great as or greater than the observed one being due to chance. If the data are grouped by combining classes N and 0 as well as classes 1, 2, and 3, so as to form a fourfold table, the P value decreases to 0.03. These low probabilities very likely are underestimates, since, in grouping, the contributions to χ^2 of the most extreme classes become excessively decreased. It appears, therefore, that the addition of the *M-4*^{3} chromosome has the effect of shifting the phenotype of *ci ey*^{R}/*ci ey*^{R} females toward normality. This result agrees with the much more striking shift toward normality of *ci ey*^{R}/*M-4*^{3} versus *ci*/o flies. The two chromosomes deficient for the *ci* region and designated as *M-4* and *M-4*^{3} thus have the same general property in regard to the *ci* phenotype. The data, however, suggest that *M-4* is more effective in contributing toward normality than *M-4*^{3}, since the differ-

ences in distribution between *ci ey^R/ci ey^R* and *ci ey^R/ci ey^R/M-4* were larger than those between *ci ey^R/ci ey^R* and *ci ey^R/ci ey^R/M-4^3* females (compare tables 7, 10, and 13 with table 11).

THE EFFECT OF THE ENTIRE FOURTH CHROMOSOME (AT 26°)

It was shown in the preceding sections of this paper that increasing numbers of the *ci* allele as well as the residue of the fourth chromosome shift the phenotype of *ci* toward normality. What is the combined effect of the two entities?

The answer to this question is already contained in the earlier results. The "addition" of the *M-4-* or *M-4^3*-chromosome to a *ci*/o and a *ci ey^R/ci ey^R* constitution (tables 7, 8, 10, 11) results in a shift toward normality, and the replacement of the *M-4* chromosome by another *ci ey^R*-chromosome leads to a further shift. The combined effect of the two entities (*ci* region and residue of the fourth chromosome) is greater than that of either alone.

THE INFLUENCE OF LOWER TEMPERATURES ON THE EFFECT OF VARIOUS DOSES OF CI AND OF THE RESIDUE OF THE FOURTH CHROMOSOME

It has been known that the penetrance of *ci* in homozygous condition is enhanced by lower temperatures (see BRIDGES 1935b). In view of certain facts regarding the effect of a normal allele of *ci*, to be discussed in a later section, it seemed desirable to obtain data on the effect of lower temperatures on the various combinations already discussed. Temperatures of 18° and 13° were chosen. In order to eliminate possible genetic diversities due to modifying genes which might have arisen in the isogenic stocks, sets of parents were kept at 26° and transferred every two days to new culture bottles. These bottles were distributed at random among 26° (control), 18°, and 13° incubators, thus neutralizing any possible effect of age of parent or environmental diversities. No significant trends due to genetic or environmental factors other than temperature were observed, with the exception of a tendency toward a more extreme *ci* phenotype in crowded bottles. Since this trend affected different constitutions similarly, it did not prove to be a disturbing factor.

A distinct effect of lowered temperature on the characteristics of *ci ey^R/ci ey^R* and *ci ey^R/M-4* flies is shown in table 12. It consists in a shift of the distribution of both phenotypes, in both sexes toward a more extreme *ci*. In regard to the small number of *ci ey^R/M-4* flies produced in the 13° transfers, it may be remarked that the limit of viability for this constitution seems to be at about this temperature. Many Minute flies did not succeed in hatching from the pupal cases. The resulting distributions are probably not seriously affected by this mortality. If any selective elimination had occurred, it would have been more likely to hit the more extreme types than the less extreme. Consequently survivors, at worst, furnish only a minimum shift.

It will be noted that the strong shift due to low temperature of the two dose constitution *ci ey^R/ci ey^R* does not "push" the distribution of *ci* phenotypes sufficiently toward the extreme as to merge it with the distribution of the one dose constitution *ci ey^R/M-4*. These latter flies themselves, though relatively

extreme at 26°, are shifted sufficiently further in expression so that the two distributions remain distinct. Only for the *ci ey^R/ci ey^R* and *ci ey^R/M-4* males, at 13°C, is the distributional difference too small to be statistically significant, but it is in the same direction as in all other cases.

In order to study simultaneously the temperature effect on the three constitutions *ci ey^R/ci ey^R*, *ci ey^R/ci ey^R/M-4*, and *ci ey^R/ci ey^R/ci ey^R*, the same type of progeny tests discussed earlier had to be made. At 18° the data (table 13) show a temperature effect, of the same kind as described in the preceding paragraphs for *ci ey^R/ci ey^R* and *ci ey^R/M-4*, on *ci ey^R/ci ey^R/ci ey^R* and *ci ey^R/ci ey^R/M-4*. All phenotypic distributions at this temperature closely

TABLE 12

F_1 of ci ey^R/ci ey^R×ci ey^R/M-4

GENOTYPE	t	♀♀ N	♀♀ 0	♀♀ 1	♀♀ 2	♀♀ 3	♂♂ N	♂♂ 0	♂♂ 1	♂♂ 2	♂♂ 3
ci ey^R/ci ey^R	26°	66	128	138	59	14	29	70	179	106	5
	18°	—	4	80	170	6	—	—	20	197	37
	13°	—	—	22	183	30	—	—	6	105	76
ci ey^R/M-4	26°	—	10	71	205	35	3	—	43	225	45
	18°	—	—	2	122	49	—	—	3	108	61
	13°	—	—	—	18	25	—	—	—	22	20

		♀♀ χ^2	♀♀ n	♀♀ P	♂♂ χ^2	♂♂ n	♂♂ P
ci ey^R/ci ey^R	26° vs. 18°	234	4	<0.01	261	3	<0.01
	18° vs. 13°	52	2	<0.01	40	2	<0.01
ci ey^R/M-4	26° vs. 18°	60	2	<0.01	42	2	<0.01
	18° vs. 13°	14	1	<0.01	2.12	1	0.1
ci ey^R/ci ey^R vs. *ci ey^R/M-4*	26°	272	4	<0.01	244	4	<0.01
	18°	107	2	<0.01	30	2	<0.01
	13°	47	1	<0.01	.68	1	0.4

approach each other, but their differences are still significant and of the same type as at 26°. At 13° an unexpected difficulty arose. The ratio of diplo- to triplo-IV *ci ey^R* flies recognized as such by progeny tests, and which was 140:98 at 26° and 96:83 at 18°, had dropped to 55:6. The low number of triplo-IV flies was compensated for, however, by 56 sterile flies (out of a total of 116 tested), while the number of sterile flies at 26° and 18° was only 24 out of 262, and 39 out of 205, respectively. Since the phenotypic distribution of the 56 sterile females from the 13° series was significantly different from that of the diplo-IV flies and within the range of that of the six recognized triplo-IV flies, the majority of the sterile flies undoubtedly represent unrecognized triplo-IV types. Thus, strangely enough, triplo-IV females raised at 13° hatch as well as diplo-IV flies but prove to be mostly sterile. Assuming the sterile females of the 13° group to be mostly triplo-IV flies, the data at this tempera-

ture become fully available for the present purpose. It is apparent that the low temperature has shifted the distributions of all three genotypes *ci ey^R /ci ey^R*, *ci ey^R/ci ey^R/M-4*, and *ci ey^R/ci ey^R/ci ey^R* toward the extreme while still retaining differences between them. The closeness of the three distribu-

TABLE 13

F_1 ♀ ♀ of ci ey^R/ci ey^R/M-4×ci ey^R/ci ey^R.
(Records of *ci ey^R/M-4* omitted.)

GENOTYPE	t	N	o	1	2	3
ci ey^R/ci ey^R/M-4	26°	14	41	148	73	1
	18°	—	1	14	149	14
	13°	—	—	1	116	28
ci ey^R/ci ey^R/ci ey^R	26°	11	36	39	12	—
	18°	—	1	17	60	5
	13°	—	—	2	3	1
ci ey^R/ci ey^R	26°	4	9	58	66	3
	18°	—	—	6	73	17
	13°	—	—	—	29	26
sterile	26°	3	6	8	7	—
	18°	—	1	7	20	4
	13°	—	—	2	46	8

		χ^2	n	P
ci ey^R/ci ey^R/M-4	26° vs. 18°	187	4	<0.01
	18° vs. 13°	18	2	<0.01
ci ey^R/ci ey^R/ci ey^R	26° vs. 18°	89	3	<0.01
	18° vs. 13°	0.02	1	0.9
ci ey^R/ci ey^R	26° vs. 18°	59	3	<0.01
	18° vs. 13°	15	1	<0.01
ci ey^R/ci ey^R/ci ey^R vs. *ci ey^R/ci ey^R/M-4*	26°	30	3	<0.01
	18°	9.06	2	0.01
	13°	17	1	<0.01
ci ey^R/ci ey^R/M-4 vs. *ci ey^R/ci ey^R*	26°	23	3	<0.01
	18°	6.18	2	0.04
	13°	15	1	<0.01

tions observed at 18° has disappeared at 13° so that the differences are more striking again.

DOSAGE EFFECTS IN TRIPLOIDS

In the summary of his dosage studies on shaven, J. SCHULTZ (1935) stated that he had begun work on *ci*. "At the moment I have only the comparison of diplo-IV cubitus interruptus triploid and diploid to offer. . . . In this case the triploid is definitely less extreme than the diploid . . ." (*l. c.*, p. 50). From this result SCHULTZ drew some far-reaching conclusions as to the nature of the

ci allele. No further account has appeared. Though the results quoted have no direct bearing on the experiments reported in the present paper, it seemed desirable to perform some tests in order to ascertain whether or not important differences exist between diploids and triploids in the effects of different doses of *ci* and of the addition of the residue of the fourth chromosome. The experiment was started with a single triploid *ci ey^R/ci ey^R/M-4* female which originated in a cross of diploid *ci ey^R/ci ey^R*×*ci ey^R/M-4*. Since the parents were isogenic, apart from the difference in the fourth chromosome, the 3 N female was likewise isogenic with those used in the other experiments. In crosses of triploid descendents of the original female, 21 *ci ey^R* triploid females were

TABLE 14

3 N ci ey^R/ci ey^R/M-4 ♀♀ × *ci ey^R/ci ey^R* ♂♂ *and other types of crosses involving 3 N females.*

	GENOTYPE	N	0	1	2	3
3 N	*ci ey^R/ci ey^R/M-4*	16	10	8	1	—
	ci ey^R/ci ey^R/ci ey^R	6	5	1	—	—
	ci ey^R/ci ey^R	—	3	3	3	—
Inters.	*ci ey^R/ci ey^R/M-4*	47	14	16	2	1
	ci ey^R (triplo-+diplo-IV)	26	13	4	—	—
2 N ♀♀	*ci ey^R/ci ey^R/M-4*	15	27	51	18	—
	ci ey^R (triplo-+diplo-IV)	10	18	36	16	—
♂♂	*ci ey^R/ci ey^R/M-4*	1	4	10	2	—
	ci ey^R (triplo-+diplo-IV)	1	5	8	4	—

	χ^2	n	P
3 N:*ci ey^R/ci ey^R M-4 vs. ci ey^R/ci ey^R ci ey^R*	0.73	1	0.4
3 N:*ci ey^R/ci ey^R* vs. *ci ey^R/ci ey^R/M-4*	3.67	1	0.06
3 N:*ci ey^R/ci ey^R* vs. *ci ey^R/ci ey^R/ci ey^R*	5.47	1	0.02
ci ey^R/ci ey^R/M-4:3 N vs. 2 N	20	2	<0.01
ci ey^R (triplo-IV and diplo-IV):3 N vs. 2 N	6.87	1	<0.01

tested and nine were determined as diplo-IV, 12 as triplo-IV. They were compared with 35 *ci ey^R/ci ey^R/M-4* triploid females from the same series of cultures (table 14). While the data are limited in number and do not show a clear trend towards a cumulative effect of three versus two *ci* alleles in the comparison of *ci ey^R/ci ey^R/M-4* with *ci ey^R/ci ey^R/ci ey^R*, they again demonstrate the effect of the fourth chromosome residue (comparison of *ci ey^R/ci ey^R* and *ci ey^R/ci ey^R/M-4*) and the combined effect of the extra *ci* and of the fourth chromosome residue as represented by a whole *ci*-containing chromosome (comparison of *ci ey^R/ci ey^R* and *ci ey^R/ci ey^R/ci ey^R*).

Any interpretation of SCHULTZ's findings is independent of the results just presented. Our data agree with his report that the triploid is less extreme than

the diploid for a given dose of *ci*. This is borne out by a comparison of triploid with diploid females both of the type *ci ey*^{R}/*ci ey*^{R}/*M-4*, and of triploids and diploids with combined groups of triplo-IV and diplo-IV. Other types of comparisons are available by including intersexes (2X3A). Intersexes approximate, and even surpass, the triploid females in their high degree of normality of *ci* phenotype. Clearly the *ci* phenotypes of intersexes do not fall between those of diploid females and males.

THE EFFECT OF THE $+^{C}$ ALLELE

The same allele, *ci*, has been dealt with throughout this discussion. The effect of the normal allele $+^{C}$, as present in the Canton-Special wild stock, alone and in combination with the *ci* allele, is now considered. (The wild type allele of *ci* would commonly be designated $+^{ci}$ but the symbol $+^{C}$ is used here to represent the particular wild type allele in the Canton-Special stock.) It is not surprising that flies homozygous for $+^{C}$ show normal phenotype at the three temperatures used. In addition to general observations accumulated in unrelated experiments, the complete normality of such flies of both sexes was specifically confirmed on 281 individuals at 26°, on 2,505 individuals at 18°, and on 435 individuals at 13°. It is indeed remarkable, however, that complete normality is not found in flies possessing only one dose of the $+^{C}$ allele (table 15). At 26° and 18° most flies of the constitution $+^{C}$/*M-4* were normal in regard to venation, though one male clearly belonged to class 0. At 13°, in both sexes, a number of flies appeared which exhibited the slight class 0 effect, and some even showed the vein interruption of class 1. In accordance with all previous experience regarding the more extreme effect of the *ci* allele in males than in females, the number of $+^{C}$/*M-4* males lacking normality is greater than that of $+^{C}$/*M-4* females. Only a few haplo-IV individuals were reared. All the 44 females reared at 26° and 18° were wild type, while one out of 52 males reared at 26° and 18° and three out of 16 males at 13° showed *ci* phenotype. This indicates a more extreme distribution in haplo-IV flies than in flies in which an *M-4* chromosome is present in addition to a normal fourth chromosome. Thus we find again a confirmation of the contribution of the *M-4* chromosome toward a normal phenotype.

These results concerning the $+^{C}$ allele provide a counterpart to the cumulative effects toward normality of *ci* alleles and their phenotypic shift away from normal at lower temperatures. The depressing action of the lower temperatures is powerful enough to make the effect of one normal allele insufficient to insure a completely normal phenotype. Instead, only a phenotype, which is usually associated with the recessive mutant allele, may be produced by the normal allele.

In addition to the individuals summarized in table 15, a number of $+^{C}$/*M-4* flies belonging to classes 0 and 1 were encountered in non-isogenic crosses. The $+^{C}$ allele, even in these cases, was derived from the Canton-Special stock. Furthermore, at least two $+^{C}$/*M-4*^{s} flies showed classes 0 and 1. It is probable that similar facts had been observed before. Thus, it had been reported (see BRIDGES-BREHME, in press) that in a stock containing *M-4* and presumably

TABLE 15

Phenotypes of flies with a single $+^c$ allele.

GENOTYPE	t	♀♀ N	♀♀ o	♀♀ 1	♂♂ N	♂♂ o	♂♂ 1
$+^c/M\text{-}4$	26°	130	—	—	126	1	—
	18°	1219	—	—	1319	—	—
	13°*	367	3	2	323	14	5
$+^c/o$	26°	36	—	—	49	1	—
	18°	8	—	—	2	—	—
	13°	—	—	—	13	2	1

* Some of these flies spent the first week of development at 18°.

not *ci*, some of the flies exhibited a *ci* phenotype. It was stated that this was a property of the *M-4* chromosome involved, which was named *M-4*^{ci}. It now appears likely either that the constitution of the "genetic background" of these flies happened to be such as to make the effect of one $+^c$ allele, in *M-4* individuals, less than normal, or that a slightly weaker normal allele than that of the Canton-Special stock was involved. The "*M-4*^{ci}" stock had died out at the time of the present work, so that this interpretation cannot be tested.

THE ANTAGONISTIC EFFECT OF CI IN COMBINATION WITH A + ALLELE

The allele *ci* has always been regarded as a recessive. Indeed, at 26° the $+^c/ci$ heterozygote has yielded exclusively normal phenotypes (table 16). Considering, however, the data of the preceding section as to the haplo-insufficiency (MOHR 1932) of the $+^c$ allele at lower temperatures, it was not surprising to find that the constitution $+^c/ci$ at low temperatures might also fall below normality. It is clear from table 16 that at 18° and especially at 13° in a considerable number of heterozygotes the effect of *ci* becomes phenotypically visible as abnormal venation.

In view of the additive effect of increased doses of *ci* on the phenotype, it is of importance to see whether an additive effect of *ci* occurs also in combination with a normal allele. If this were true, the phenotypic distribution of $+^c/ci$

TABLE 16

Phenotypes of $+^c+^w/ci\ ey^R$ or $+^c/ci$ flies.

t	♀♀ N	♀♀ o	♀♀ 1	♂♂ N	♂♂ o	♂♂ 1
26°	1081	—	—	960	—	—
18°	892	1	—	805	19	—
13°*	1132	40	4	884	164	17

* Some of these flies spent the first week of development at 18°.

as compared to that of $+^{c}/M\text{-}4$ should be shifted toward normality. In order to test this point, both types of flies were raised together at 13° in the same cultures (table 17). Taking the results at their face value, it appears that many more heterozygous than hemizygous flies deviate from normality. Statistically this difference has a probability of 0.09 for the females to be due to chance; for the males its significance is beyond doubt.

This astonishing result seems to indicate not an additive effect of *ci* but an effect antagonistic to that of $+^{c}$. The data, however, are subject to another interpretation. While the differences in distribution are due to greater relative frequency of class 0 in $+^{c}/ci$ as compared with $+^{c}/M\text{-}4$, class 1 was equally frequently represented in both genotypes. It appears that the distributions of

TABLE 17

F$_1$ *of* $+^{c}+/+^{c}+ \times ci\ ey^{R}/M\text{-}4$. 13°.

GENOTYPE	♀♀ N	♀♀ 0	♀♀ 1	♂♂ N	♂♂ 0	♂♂ 1
$+^{c}+/ci\ ey^{R}$	621	21	3	493	100	12
$+^{c}/M\text{-}4$	297	3	2	232	12	5

		χ^2	n	P
$+^{c}+/ci\ ey^{R}$ vs. $+^{c}/M\text{-}4$	♀♀	2.96	1	0.09
$+^{c}+/ci\ ey^{R}$ vs. $+^{c}/M\text{-}4$	♂♂	18	1	<0.01

the two genotypes are not simply shifted as wholes but that they are not strictly comparable due to the disproportionately large decrease of class 0 among $+^{c}/M\text{-}4$. The cause for this decrease is perhaps connected with the thinness of wing veins in Minute as compared to non-Minute flies, so that slight thinning of veins which is visible in the latter phenotype becomes unrecognizable in Minute types. Whatever the cause, no reliable conclusion can be drawn from the data.

In order to study the effect of adding a *ci* allele to a normal allele without the secondary phenotypic difference between Minute and non-Minute flies, a comparison was made between the phenotypes of $ci/+/M\text{-}4$ and $ci/+/ci$, both of which are non-Minute. For methodologic purposes the stock containing the + allele was chosen so as to contain the mutant ey^{2}. The normal allele of *ci* present in this stock is of different origin than that in the Canton-Special stock. It will be referred to as $+^{2}$. In order to obtain the desired genotypes, crosses were made, at 13°, between $ci\ ey^{R}/ci\ ey^{R}/M\text{-}4$ and $+^{2}\ ey^{2}/+^{2}\ ey^{2}$ parents; the latter were not isogenic with the majority of flies used (for a check of the non-significance of this lack of isogeneity see p. 467). Three phenotypically distinguishable classes of offspring originate from this cross—namely, non-Minute, non-eyeless flies of the constitution $ci\ ey^{R}/M\text{-}4/+^{2}\ ey^{2}$, non-Minute, eyeless flies of either $ci\ ey^{R}/+^{2}\ ey^{2}$ or $ci\ ey^{R}/ci\ ey^{R}/+^{2}\ ey^{2}$, and Minute flies $+^{2}\ ey^{2}/M\text{-}4$. The eyeless flies were assigned, by means of progeny

tests, to one or the other of the two genotypes named (table 18). These progeny tests, carried on with males only, may be regarded as reliable, since the distribution of the sterile flies does not deviate significantly from the sum of the tested fertile flies. All genotypes given in table 18 contain the normal $+^2$ allele of *ci*, but nevertheless exhibit the mutant *ci* phenotype in a large percentage of individuals. This is a confirmation of the data derived from combinations with the $+^C$ allele. On the other hand, a difference between the present and the earlier data is apparent in the much higher relative frequency of flies with *ci* phenotype in both *ci* $ey^R/+^2$ ey^2 and $+^2$ ey^2/M-*4* classes than

TABLE 18

F_1 of ci ey^R/ci ey^R/M-4×$+^2$ ey^2/$+^2$ ey^2.

(a)	*ci* ey^R/*M-4*/$+^2$ ey^2				*ci* ey^R/$+^2$ ey^2 plus *ci* ey^R/*ci* ey^R/$+^2$ ey^2					$+^2$ ey^2/*M-4*				
	N	o	1	2	N	o	1	2	3	N	o	1	2	3
♀♀	1201	45	9	1	1364	163	149	—	3	438	30	27	12	—
♂♂	952	98	20	1	926	365	141	21	1	286	35	38	27	1

(b)		N	o	1	2	3
Progeny tests of 502 males of the above group "*ci* $ey^R/+^2$ ey^2 plus *ci* ey^R/*ci* $ey^R/+^2$ ey^2" yielded	*ci* $ey^R/+^2$ ey^2	240	63	11	—	—
	ci ey^R/*ci* $ey^R/+^2$ ey^2	40	45	26	3	—
	sterile	43	23	7	1	1

♂♂	χ^2	n	P
ci $ey^R/+^2$ ey^2 vs. *ci* ey^R/*ci* $ey^R/+^2$ ey^2	77	2	<0.01
ci $ey^R/+^2$ ey^2 plus *ci* ey^R/*ci* $ey^R/+^2$ ey^2 vs. sterile	1.83	2	0.4
ci $ey^R/+^2$ ey^2 vs. $+^2$ ey^2/*M-4*	36	2*	<0.01
ci ey^R/*M-4*/$+^2$ ey^2 vs. *ci* $ey^R/+^2$ ey^2	32	2	<0.01
ci ey^R/*M-4*/$+^2$ ey^2 vs. *ci* ey^R/*ci* $ey^R/+^2$ ey^2	218	1	<0.01
$+^2$ ey^2/*M-4* vs. *ci* ey^R/*ci* $ey^R/+^2$ ey^2	15	2*	<0.01

* Classes N and o were combined; see text.

was met with in flies containing $+^C$. A comparison between the distribution within the heterozygous and hemizygous group presents another difference. In combinations with $+^C$ class 0 was four times more frequent in $+^C/ci$ than in $+^C/M$-*4* males, while the poorly represented class 1 was about equal in both constitutions. In contrast, in combination with $+^2$, class o was more than twice as frequent in $+^2/ci$ than in $+^2/M$-*4*, and the more extreme classes 1 and 2 were well represented among the $+^2/M$-*4* group and outnumbered greatly the corresponding classes of $+^2/ci$. If one admits the unreliability of classification 0 for a comparison between Minute and non-Minute flies and therefore combines classes N and 0 for the statistical treatment, it is seen that *ci* $ey^R/+^2$ ey^2 males deviate significantly less from normality than do

$+^2$ *ey*^{2}/*M-4* males. Thus the addition of the *ci* allele to the latter constitution has tended to shift the phenotype toward normal, and the formerly established cumulative effect of the *ci* allele in combination with other *ci* alleles has been shown to be true also for the combination with the $+^2$ allele, as represented by *ci ey*^{R}/$+^2$ *ey*^{2}. (Whether the differences between the phenotypes of flies carrying the $+^C$ allele and those carrying $+^2$ are primarily due to differences in the two + alleles, or whether the different genetic background is responsible will not be investigated at this place.)

A confirmation is further obtained for the property of the *M-4* chromosome residue to shift the *ci* phenotype toward normal, as seen in the distribution of *ci ey*^{R}/*M-4*/$+^2$ *ey*^{2} as compared with that of *ci ey*^{R}/$+^2$ *ey*^{2} males.

The most important comparison, that of *ci ey*^{R}/*M-4*/$+^2$ *ey*^{2} and *ci ey*^{R} /*ci ey*^{R}/$+^2$ *ey*^{2} flies provides a reversal of the relations hitherto met with. The latter group, beyond doubt, is very much less normal phenotypically than the former. It is obvious that the "addition" of the *ci* allele to the *ci ey*^{R}/*M-4* /$+^2$ *ey*^{2} genotype has not resulted in a cumulative effect of the alleles of the *ci* locus toward normality, but has, to the contrary, produced an antagonistic effect. This antagonistic effect is even apparent in a comparison of *ci ey*^{R}/$+^2$ *ey*^{2} and *ci ey*^{R}/*ci ey*^{R}/$+^2$ *ey*^{2}. The addition of a whole *ci ey*^{R} chromosome in the latter genotype as compared to the former represents not only an addition of *ci* to the heterozygote but also an addition of the chromosome residue. Although the residue by itself was shown to be effective toward normality, the joint effect of the combination with *ci* leads to greater abnormality in the *ci ey*^{R}/*ci ey*^{R}/$+^2$ *ey*^{2} group as contrasted with *ci ey*^{R}/$+^2$ *ey*^{2}. Finally, the antagonistic effect of the *ci ey*^{R} chromosome in certain combinations with $+^2$ is seen strikingly in a comparison of $+^2$ *ey*^{2}/*M-4* and *ci ey*^{R}/*ci ey*^{R}/$+^2$ *ey*^{2}. While the replacement of the *M-4* chromosome by one *ci ey*^{R} chromosome was shown to lead to a phenotypic shift toward normality, the replacement of *M-4* by two *ci ey*^{R} chromosomes results in a significantly greater degree of abnormal *ci* venation.

TABLE 19

F$_1$ *males of ci ey*^{R}/*ci ey*^{R} ♀♀ *(containing both sets of first, second and third chromosomes derived from the* $+^2$ *ey*^{2} *stock)*×*ci ey*^{R}/*ci ey*^{R}/*M-4* ♂♂ *(from the usual isogenic stock).*

	N	0	1	2
ci ey^R/*ci ey*^{R}/*M-4*	—	7	80	88
ci ey^R/*ci ey*^{E}/*ci ey*^{R}	3	3	19	24
ci ey^R/*ci ey*^{R}	—	2	33	79
sterile	—	4	15	15

	x^2	n	P
ci ey^R/*ci ey*^{R}/*M-4* vs. *ci ey*^{R}/*ci ey*^{R}/*ci ey*^{R}	6.72	1	0.01
ci ey^R/*ci ey*^{R}/*ci ey*^{R} vs. *ci ey*^{R}/*ci ey*^{R}	6.02	1	0.01
ci ey^R/*ci ey*^{R}/*M-4* vs. *ci ey*^{R}/*ci ey*^{R}	10	1	<0.01
ci ey^R/*ci ey*^{R} plus *ci ey*^{R}/*ci ey*^{R}/*ci ey*^{R} vs. sterile	4.21	1	0.04

The results are so singular that it seemed necessary to safeguard against the slight possibility of the non-isogenic background in this cross being responsible for them. However, a comparison of *ci ey^R/ci ey^R/ci ey^R* with *ci ey^R/ci ey^R/M-4* and with *ci ey^R/ci ey^R* conducted on the same genetic background as that represented in table 18, gave the usual phenomenon of cumulative action of *ci* alleles (table 19. It is doubtful whether the sterile flies in this experiment are a random sample of the joint population of *ci ey^R/ci ey^R* and *ci ey^R/ci ey^R /ci ey^R*. The distribution of the sterile flies agrees more closely with that of the *ci ey^R/ci ey^R/ci ey^R* group. This suggests that the majority of the sterile flies may have been of the *ci ey^R/ci ey^R/ci ey^R* genotype. Had they been fertile, the significance of the differences in distribution between the three fertile groups would have been enhanced). In summary, the antagonistic effect of *ci* in certain combinations with the normal allele $+^2$ in contrast to the cumulative effect of multiple doses of the *ci* allele alone was not due to a special genetic background but must be regarded as typical.

POSSIBLE DIFFERENCES IN CLASS DISTRIBUTIONS IN RECIPROCAL CROSSES

In several series of experiments summarized in the tables of this paper reciprocal crosses between the two parental types were made. Their outcome suggests differences in the distributions among the classes of *ci* phenotypes, but since the general trend in comparisons was not affected, no separate tabulations are presented at this time. Further data are being accumulated.

INTERPRETATION

The action of the normal and mutant alleles at the ci locus

An interpretation of the relationship between gene dosage and phenotype may begin with the postulate that genic action is dependent in its initial step on a reacting material present in the immediate nuclear surroundings of the gene. This reacting material will be called the substrate (S). The material resulting from the interaction between gene and substrate will be called the gene product (P). It will further be assumed that different amounts of P are so correlated with phenotypic effect that the effect either increases with increasing P or, having reached a maximum, remains constant. This latter assumption has a factual basis in so far as it has been found that different doses of either the *ci* allele or of the $+^C$ allele are correlated with the observed phenotypic effect in such a way that the latter increases with increasing number of alleles, or, having reached a maximum, remains constant.

It has been shown that at 26°, 18°, and 13° an increase of the number of *ci* alleles from one to two in diplo-IV flies and from one to two and two to three in triplo-IV flies leads to a closer approach to normality. It may be concluded that at 26°, 18°, and 13°

(1a) the *ci* allele acts similarly but less effectively than a + allele

(1b) the amount of substrate available in a diplo-IV nucleus is in excess of that utilized by one *ci* allele

(1c) the amount of substrate available in a triplo-IV nucleus is in excess of that utilized by two *ci* alleles.

It has further been shown that, at 13°, one $+^{c}$ allele, or one $+^{2}$ allele, is not sufficient to lead to a completely normal phenotype, while two $+^{c}$ alleles are always able to do so. From this it may be concluded that at 13°

(2a) the alleles $+^{c}$ and $+^{2}$ act similarly but more effectively than the *ci* allele (this is an independent confirmation of conclusion (1a) above)

(2b) the amount of substrate available in a diplo-IV nucleus is in excess of that utilized by one $+^{c}$ allele.

It is not known whether at the higher temperatures the normality of phenotypes resulting from one as well as from two $+^{c}$ alleles is a consequence of a limitation of substrate relative to the reacting capacity of the $+^{c}$ allele, so that one allele is able to form as much P as two, or whether in spite of a difference in amount of P formed, the product of one allele is sufficient to lead to full normality. In other words, it is unknown whether temperature affects the primary genic reaction leading to P or a later secondary one.

The differences in phenotypes which lead to conclusions (1b), (1c), and (2b) permit also a deduction as to the secondary reactions which follow the primary interaction of gene+substrate→P. It would not have been possible to discover differences in P were it not that these differences led to differences in all later reaction products, P', $P'' \cdots P^{n}$. Therefore, for the genotypes and respective temperatures considered in (1) and (2), it may be concluded that

(3) no secondary reaction, in the course of development has a threshold which cancels differences of the primary product P, which is in turn due to differences in the quantity of the *ci* or $+^{c}$ alleles.

Perhaps the most striking experimental result relates to the effects at 13° of the combination of the $+^{2}$ with two *ci* alleles as compared with the effect of the combination of a $+^{2}$ allele and a single *ci* allele. It was found that the addition of the *ci* allele to the $+^{2}/ci$ combination brought about a decrease in capacity to form normal venation, in contrast to the fact that the addition of the *ci* allele to other *ci* alleles leads to increased capacity. It may be well to stress the fact that in both types of experiments exactly the same addition was made. Stated more accurately, it consisted in the latter experiments of the replacement in *ci* $ey^{R}/M\text{-}4$ or *ci* ey^{R}/ci $ey^{R}/M\text{-}4$ flies of the *M-4* chromosome by a *ci* ey^{R} chromosome, and in the former experiments, of the replacement, in $+^{2}$ ey^{2}/ci $ey^{R}/M\text{-}4$ flies, of the *M-4* chromosome by the *ci* ey^{R} chromosome. In a very abstract way it may be concluded that the *ci* allele possesses both positive and negative qualities in regard to the capacity to participate in normal vein formation. The positive capacity outweighs the negative if only *ci* alleles are present in the nucleus while the negative capacity is paramount if in the given combination a $+^{2}$ allele is also present in the nucleus. In a less abstract way two properties may be assigned to each allele, a combining power (c) and an efficiency factor (e). Combining power will be defined

as power to react with the substrate S. The two alleles $+^2$ and *ci* may have different c powers, c_{+^2} and c_{ci}, so that the amount of S utilized by the two alleles is different. On the basis of the present data no conclusion has been reached regarding the relation of c_{+^2} and c_{ci}—that is, it is unknown which allele has a higher combining power. The efficiency factor will be defined so as to include two different but related concepts. It may be assumed that the $+^2$ and *ci* alleles interact with the substrate so as to form an identical product P or two different products, P_{+^2} and P_{ci}, but of equal effectiveness in forming the final phenotype. In this case the efficiencies e_{+^2} and e_{ci} signify the relative amount of P formed by the allele per unit of substrate used. On the other hand, it may be assumed that the two alleles form different products, P_{+^2} and P_{ci}, which possess different effectiveness in producing the final phenotype. In this case the efficiency factors e_{+^2} and e_{ci} signify the relative amount of P_{+^2} or P_{ci} formed per unit of substrate used and multiplied by a constant indicating the relative effectiveness of the different products. This latter definition of e_{+^2} and e_{ci} includes the earlier one as a limiting case. The latter, more general definition will form the basis of the following discussion. If the efficiency of the $+^2$ and *ci* alleles were equal, no antagonistic result from the addition of *ci* to $+^2$ could occur. If, however, the efficiency of *ci* is lower than that of $+^2$ ($e_{ci} < e_{+^2}$), and if the amount of substrate, S, is limited to an amount below that which *ci* and + together can utilize, then the competition for S between the two kinds of alleles of the heterozygote $+^2/ci/ci$ will result in the $+^2$ gene's not obtaining enough S to produce its maximum effect, while the *ci* genes will use their fraction of S less efficiently than $+^2$. Hence the limited amount of S present will be utilized less efficiently by the cooperation of $+^2$ and the two *ci* alleles than by the combination of $+^2$ and one *ci* allele. Both the cumulative and antagonistic effects of varying doses and combinations of the alleles at 13° are thus explained if it is postulated that

(4a) the amount of substrate available in a triplo-IV nucleus is below the joint combining powers of the one $+^2$ and two *ci* alleles

(4b) the efficiency of the *ci* allele in converting S into a product effective toward producing a normal phenotype is lower than that of the $+^2$ allele.

The concept of combining and efficiency factors as properties of genic action furnishes a general understanding of the observation that there are sometimes at the same locus different mutant alleles, some of which are recessive and others dominant when present in combination with the normal allele. This situation is known in various organisms as, for example, *Zea mays* and *Drosophila melanogaster*, and in the latter including the *ci* locus where, at 26°, the *ci* allele is recessive while two other alleles, known as ci^W and ci^D are dominant, to varying degrees. A dominant mutant allele, in such a case, may be regarded as one whose combining power is large enough so that competition for the substrate exists between the normal and the mutant allele, and whose efficiency is low enough to reduce the effective sum of the products P below the amount necessary for normality.

The general trend of the foregoing analysis is related to earlier studies of the author (1929, 1930) and owes much to the work of GOLDSCHMIDT (1927, 1938), MOHR (1932), MULLER (1932), and especially WRIGHT (1934, 1941a, b). The well known terminology "hypomorph," "antimorph," etc., has been avoided, since it suggests, unintentionally, a linear relationship of the activities of various alleles. On the contrary, the proposed scheme makes use of a two-dimensional system of gene properties. Were they not too inconvenient, the terms "hypo-combining, hypo-efficient," "hypo-combining, homo-efficient," etc., would be descriptive of the relation of various alleles to each other.

The assumption of competition of different alleles of a gene for a common substrate is in line with a prevalent hypothesis according to which different alleles of a gene are chemical units of similar configuration. It is becoming known increasingly that compounds of highly similar chemical constitution may interfere with each other with respect to their effect on living cells—that is, they may compete, with different efficiency, for a limited cellular substrate.

The effect of the fourth chromosome residue

It has been shown that, at 26°, the main body of the fourth chromosome, exclusive of the region which includes the *ci* locus, acts like the *ci* gene in shifting the *ci* phenotype toward normality. At least two interpretations of this fact are possible. It may be that genes in the *M-4* and *M-4*^{3} chromosomes happen to affect secondary stages in the development of wing venation so as to diminish to some degree the disturbances due to *ci*. If this were so, both the *M-4* and *M-4*^{3} chromosomes would be nothing but carriers of genes modifying a *ci* phenotype in the same direction. An alternative interpretation might assume that the similarities of action of these chromosomes, to each other and to that of the *ci* locus, are not coincidences but that it is a typical function of the main body of the fourth chromosome to contribute toward a normal phenotype by furnishing substrate for the action of the *ci* locus. This second interpretation appears preferable, since it assigns an inherent cause for the observed effects. Furthermore, it fits in with the fact that the effect of the *ci* locus is very frequently changed when disturbed in its position in the normal fourth chromosome (DUBININ and SIDEROFF 1934; STERN and HEIDENTHAL, in preparation). Since the position effect of a translocated *ci* locus may be interpreted, in general, as due to reduction in available substrate, it follows that this reduction is due to either the presence of new neighboring chromosome regions or to the absence of the old ones, or to both. The high proportion of translocations near the *ci* locus which show a position effect suggests that absence of the normal neighborhood, as represented by the main body of the fourth chromosome, plays an important rôle. Obviously these considerations cannot be regarded as more than suggestive. Moreover, they leave unexplained the fact that the *M-4*^{3} chromosome seems weaker in its effect than the *M-4* chromosome, though the deficiency in both of them is stated to be alike. We are investigating at present whether the two deficiencies are really indistinguishable in salivary gland chromosomes.

The effect of other than the fourth chromosomes

A problem similar to that regarding the type of action of the fourth chromosome residue arises in considering the effect of genetic differences due to change of quantities of the X chromosomes and of the large autosomes. It has been shown that the phenotype of *ci* females is shifted toward normality if compared with *ci* males, and that triploid females and intersexes are still more normal. Again, the increase in chromosome material within the nucleus may be effective by supplying more of the substrate on which the *ci* locus may act. Considering that the substrate itself may be the resultant of the interaction of many genes, such a hypothesis is not impossible. On the other hand, it is comparatively more likely in these cases that the effect on *ci* is due to secondary, modifying action. Diploid males and females and triploid females and intersexes all are developmentally and phenotypically so different that the *ci* reaction series obviously occurs in very different backgrounds, whereas, by contrast, there are almost no phenotypical differences between the diplo-IV and triplo-IV flies of any one of the four different sex types.

The effect of different temperatures

The environmental effect of lowered temperature poses again the problem of the site of action of agents other than the *ci* locus on the *ci* phenotype. Before discussing the general problem, it may be pointed out that it is not justified to think of different temperatures exclusively as direct modifiers of the *ci* reaction relative to other developmental reactions. It is possible, for instance, that different temperatures act by changing the population of microorganisms in the food, or the metabolic activities of these organisms, and thus indirectly alter the development of Drosophila. Another factor, body size, whose frequent significance has recently been demonstrated by NEEL (1940), is hardly of major import in the case of *ci*. While the more extreme phenotype of *ci* males as compared to females may suggest a positive correlation between body size and degree of vein normality, the temperature data present a negative correlation. At the lower temperatures larger flies are produced but with less normal venation than at higher temperatures.

The present studies, which combine temperature and dosage experiments, were undertaken in an attempt to test aspects of the postulated relation between the *ci* gene and substrate. A more extreme *ci* phenotype at lowered temperature may be explained in various ways at least one of which is relevant to the data obtained. This hypothesis would assume the presence of a lowered amount of available substrate relative to the combining power of *ci*. If this were the cause, an excess of substrate found to exist at 26° might be reduced sufficiently at the lower temperatures so as to make the amount of substrate a limiting factor. More specifically, it might be expected that with this limitation in S, three *ci* alleles together might have hardly more effect than two or possibly even one *ci* allele. Such disappearance of the dosage effect at lower temperatures should result from a kind of telescoping shift of phenotypes, those due to three *ci* doses being more affected than those due to two doses,

and those based on two doses more affected than those due to one single dose. Moreover, the medium temperature of 18° would be expected to lead to phenotypical shifts in the genotypes with larger doses only, while the lowest temperature (13°) might affect even the genotypes with smaller *ci* doses. These expectations follow from the assumption of decreasing amounts of S at lower temperatures, since an amount low in proportion to c_{ci} would become more strongly apparent in the reaction with three c_{ci}, than with two c_{ci} and least, or perhaps not at all, with one c_{ci}. This interpretation of the effect of lowered temperature on the *ci* reaction can be confronted with the data obtained at 18° and 13°. They show that shifts of the distributions toward a more extreme phenotype than at 26° occurred in all genotypes, including that with only one *ci* allele, and that these shifts, in all genotypes, were greater at 13° than at 18°. It was noted that at 18° triplo-IV flies with three and two alleles resembled each other rather closely. However, at 13° the differences in phenotypic distribution were again—as at 26°—more striking than at 18°, suggesting that the similarities at 18° were rather the result of the coarse type of phenotypic classification employed than of intrinsic causes related to gene-substrate interaction.

Since each of the different genotypes participates in the phenotypical shifts, it may be concluded that the effect of lowered temperatures is not due primarily to reducing the substrate below a level in excess of the combining power of the different doses of *ci* alleles. This does not imply that temperature has been shown to affect secondary *ci* reactions. Rather it is left for the future to decide whether the phenotypic shifts are caused mainly by temperature influencing in some other way the gene-substrate interaction, or whether the shifts are due to later developmental interrelations.

CONCLUSION

In this paper conclusions have been reached relating to two different levels. In the main part, observations are recorded in regard to the phenotypic effects of various genotypes, under varied environmental conditions. It is shown that increase in number of *ci* alleles in flies in which no other allele of this locus is present leads cumulatively to more nearly normal phenotypes. On the other hand, in certain circumstances, the effect of a normal allele may not result in a normal, but in a *ci* phenotype. It is shown further that the cumulative action of the *ci* allele may become reversed into an antagonistic one if *ci* is in combination with a + allele. In these cases the "recessive" *ci* allele becomes a "dominant." Other effects on the phenotypes are observed if the *ci* locus is held constant while the dose of the remainder of the fourth chromosome is changed.

In the second part of the paper a theoretical interpretation is proposed which relates the phenotypic effects to primary interaction of gene and gene substrate. The data may have significance as they stand; but the interpretation, it is realized, may represent no more than a passing attempt.

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SUMMARY

An increase from one to two doses of the fourth chromosome mutant allele cubitus interruptus (*ci*) of *Drosophila melanogaster*, in diplo-IV flies, produces a more normal phenotype of the cubital vein. Similarly, an increase from one to two and from two to three *ci* alleles in triplo-IV flies builds up toward a normal phenotype.

The dosage effect of *ci* is a true one, independent of environmental conditions, such as hatching order, or of genetic marker genes.

The dosage differences of *ci* were made possible by the use of the *M-4* chromosome deficient for a short region which includes the *ci* locus. If the number of *M-4* chromosomes is varied from none to two, while the number of *ci* containing chromosomes is kept constant, a shift toward a normal vein phenotype is observed. Thus, the *M-4* chromosome, designated as the "residue" of the fourth chromosome, has an effect similar to that of *ci*. Another residue chromosome (*M-4*^{3}), apparently deficient for the same section as *M-4*, likewise shifts the *ci* phenotype toward normal though perhaps to a lesser degree than *M-4*.

The joint effect of addition of *ci* doses and of fourth chromosome residues is greater than that of either addition alone.

Decrease of temperature, from 26° to 18° and 13° leads to shifts of all phenotypes toward more extreme abnormality. Phenotypic differences due to different genotypes seem to persist in spite of the shifts.

The dosage effects described above for diploids can also be demonstrated in triploids. The same constitution with regard to the fourth chromosome produces a more normal phenotype in triploid females and intersexes than in diploid females. The latter in turn are more normal than males.

Two normal alleles of *ci*, derived from the Canton wild stock, produce a normal phenotype at all temperatures tested. One single $+^C$ allele, or one single $+^2$ allele derived from the *ey*^{2} stock, particularly at 13°, may not be able to lead to normality but may cause a weak *ci* phenotype. Whether $+^C$ and $+^2$ are different alleles awaits further data.

The heterozygotes $+^C/ci$ and $+^2/ci$ at 26° show dominance of +; at lower temperatures, *ci* becomes slightly dominant.

Heterozygous $+^2/ci/ci$ individuals show a more extreme degree of *ci* phenotype than $+^2/ci$ individuals. Therefore, the addition of *ci* to $+^2/ci$ detracts from the joint action of $+^2/ci$ toward normality, in contrast to the addition of *ci* to *ci* or *ci*/*ci* which acts cumulatively toward normality.

A theory of primary genic interaction with a nuclear substrate, S, is developed. The data become intelligible if at least two properties are ascribed to each allele, (1) a combining power, designating the degree of interaction with S, and (2) an efficiency factor measuring the effectiveness of interaction in forming a product P efficient in the elaboration of a normal phenotype.

The theory leads to statements as to excess or limitation of S relative to the combining powers of the *ci* and + alleles in various genotypes (cf. conclusions 1a–c, 2a–b, and 4a, pp. 467–469).

The efficiency of the *ci* allele is smaller than that of + (cf. 4b, p. 469).

Differences in the amount of P resulting from the primary reaction are not cancelled by thresholds involving secondary reactions (cf. 3, p. 468).

The effect of the residue of chromosome IV may be due to its furnishing S for the *ci* locus.

The effect of lower temperatures on the *ci* phenotype does not seem to consist in reducing S.

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THE GENETIC BASIS FOR CONSTRUCTING SELECTION INDEXES[1]

L. N. HAZEL[2]
Iowa State College, Ames, Iowa

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"The key is man's power of accumulative selection: nature gives successive variations; man adds them up in certain directions useful to him."—Darwin, p. 35, sixth edition of *The Origin of Species*. 1920.

INTRODUCTION

THE idea of a yardstick or selection index for measuring the net merit of breeding animals is probably almost as old as the art of animal breeding itself. In practice several or many traits influence an animal's practical value, although they do so in varying degrees. The information regarding different traits may vary widely, some coming from an animal's relatives and some from the animal's own performance for traits which are expressed once or repeatedly during its lifetime. LUSH (1935) emphasized that permanent improvement from phenotypic selection is proportional to the additively genetic (heritable) fraction of the observed variance and that this varies for different traits. DOBZHANSKY (1937) suggested "that most, and possibly all, genes have manifold effects." These factors make wise selection a complicated and uncertain procedure; in addition fluctuating, vague, and sometimes erroneous ideals often cause the improvement resulting from selection to be much less than could be achieved if these obstacles were overcome.

In the initial stages of breeding investigations conducted by the REGIONAL SWINE BREEDING LABORATORY and cooperating state experiment stations, an arbitrary method of selecting breeding animals had to be adopted. In the meantime the theoretical aspects of the problem were investigated while data were being collected. While many fundamental genetic problems are still incompletely solved (particularly as regards the prevalence of dominance, epistasis and pleiotropic effects of genes in quantitative inheritance, the nature of heterosis, and the interaction of genotype and environment), the accumulation of genetic knowledge justifies an exploration of this problem. According to formulas presented by HAZEL and LUSH (1943), selection for an index which gives proper weight to each trait is more efficient than selection for one trait at a time or for several traits with an independent culling level for each trait. The principles of constructing and using selection indexes which permit the attainment of maximum genetic progress are given in the present paper. Three selection indexes were constructed (and compared as to efficiency) from data taken on the Iowa Station swine herd from the fall of 1937 through the spring of 1940. Such indexes are subject to revision when the accuracy of

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[2] Now at the WESTERN SHEEP BREEDING LABORATORY, Dubois, Idaho.

the statistics upon which they are based can be increased by analyzing additional data.

Selection indexes, constructed with attention to the genetic and economic bases for the various traits, should be valuable in livestock breeding programs. GALTON'S "Law of Regression," presented before the Mendelian nature of inheritance was clear, represents an early step in index construction. The sire index, widely used in selecting sires for butterfat and milk production, is a practical example of an index based on one trait but using information about several relatives. SMITH (1937) developed an index designed for the selection of plant lines, using FISHER'S concept of discriminant functions to derive a linear equation based on observable characteristics as the best available guide to the genetic value of each line.

ANALYTICAL METHOD

The net genetic improvement which can be brought about by selecting among a group of animals is the sum of the genetic gains made for the several traits which have economic importance. It is logical to weight the gain made for each trait ($\overline{G}_i$) by the relative economic value of that trait (a_i). Thus the average genetic superiority of a selected group over the group from which it was chosen is

$$\overline{H} = a_1\overline{G}_1 + a_2\overline{G}_2 + \cdots + a_n\overline{G}_n. \tag{1}$$

The relative economic value for each trait depends upon the amount by which profit may be expected to increase for each unit of improvement in that trait. Good approximations to relative economic values often can be obtained from long-time price averages and cost-of-production figures. As an example, WINTERS (1940) found that the average price of wool per pound was 3.4 times that of lamb per pound. If additional feed or labor costs are associated with increased production for either trait, the increased cost per unit should be discounted when calculating relative economic values. These values may vary from breed to breed or from region to region within the same breed. They may change, even while a breeding program is in progress, if permanent shifts in market demand occur.

Animals vary in breeding value, as in phenotype, for each of the several traits. The aggregate value of an animal is the sum of its several genotypes (assuming a distinct genotype for each economic trait), each genotype being weighted according to the relative economic value of that trait. An animal's genotype for a given trait may be defined as the sum of the average (strictly additive) effects of its genes which influence that trait. Thus the aggregate genotype of an animal is

$$H = a_1G_1 + a_2G_2 + \cdots + a_nG_n. \tag{2}$$

Environmental factors, dominance, and epistasis may make phenotypic performance unlike the genotype for that trait; hence animals having the highest values for H cannot be recognized directly with perfect accuracy. Selection for improved breeding value therefore must be practiced indirectly by select-

ing directly for a correlated variable (I) based on the phenotypic performance of each animal for the several traits. When selection is practiced on a large population, the genetic average of the selected group minus that of the original population represents the genetic gain from selection ($\overline{H}$ in equation 1), being

$$\overline{H} = (i)R_{IH}\sigma_H. \tag{3}$$

Here $\bar{i}$ is the average superiority of the indexes (in standard deviation units) for the selected group as compared to the whole group, R_{IH} is the correlation between H and I, while σ_H is the standard deviation of H in the whole group.

The selection differential ($\bar{i}$) depends upon the proportion which can be culled, being limited by the percentage needed for replacements for a particular species. The standard deviation of breeding value,

$$\sigma_H = \sqrt{a_1^2\sigma_{G_1}^2 + a_2^2\sigma_{G_2}^2 + \cdots + 2a_1a_2\sigma_{G_1}\sigma_{G_2}r_{G_1G_2} + \cdots},$$

depends upon gene frequency and to some extent upon the mating system. These can be changed only a little by the breeder. Consequently the opportunity for increasing the progress expected from selection lies in making R_{IH} as large as possible. Accordingly I is defined as

$$I = b_1X_1 + b_2X_2 + \cdots + b_nX_n, \tag{4}$$

where the X's represent phenotypic performance for the several traits and the b's are multiple regression coefficients chosen so as to make R_{IH} as large as possible. These regression coefficients may be calculated from n simultaneous equations

$$\begin{aligned}
&\beta_1 + \beta_2r_{X_1X_2} + \cdots + \beta_nr_{X_1X_n} = r_{X_1H}\\
&\beta_1r_{X_1X_2} + \beta_2 + \cdots + \beta_nr_{X_2X_n} = r_{X_2H}\\
&\cdots\cdots\cdots\cdots\cdots\cdots\\
&\beta_1r_{X_1X_n} + \beta_2r_{X_2X_n} + \cdots + \beta_n = r_{X_nH}
\end{aligned} \tag{5}$$

where $\beta_i = b_i\dfrac{\sigma_{X_i}}{\sigma_H}$ and r_{X_iH} is the correlation between H and the i-th phenotypic measurement.

The simultaneous equations can be solved only if estimates of the various correlations can be calculated. The usual methods of interclass and intraclass correlation are generally sufficient to calculate the phenotypic correlations ($r_{X_iX_j}$). WRIGHT'S (1934) method of path coefficients is convenient for calculating the more complex correlations between H and phenotypic performance (r_{X_iH}). The path coefficient diagram (fig. 1) indicates the various relations between H and the phenotypic measurement for each trait (X_i). This correlation is the sum of the various paths from X_i to H, as follows,

$$r_{X_iH} = r_{G_iX_i}\{d_1r_{G_iG_1} + d_2r_{G_2G_i} + \cdots + d_nr_{G_nG_i}\}, \tag{6}$$

where $$d_i = a_i \frac{\sigma_{G_i}}{\sigma_H}.$$

Therefore we must have estimates of genetic variability for each trait (σ_{G_i}), the correlation between genotype and phenotypic performance for each trait ($r_{X_iG_i}$), and the correlation between genotypes for different traits ($r_{G_iG_j}$) to solve the simultaneous equations.

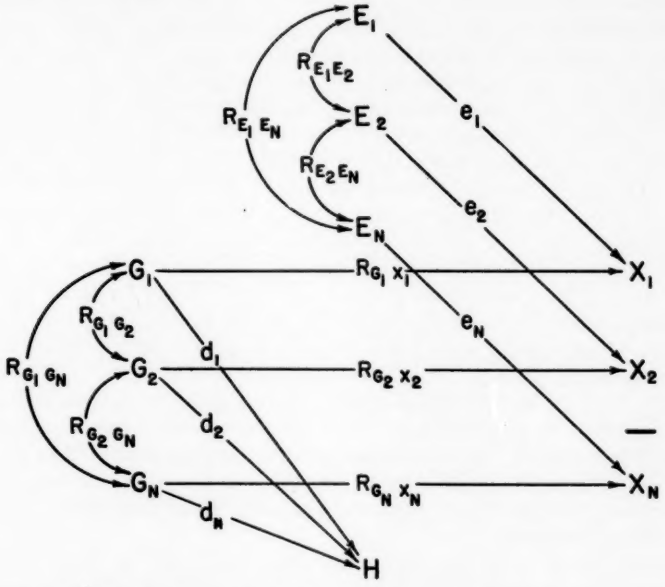

FIGURE 1. Path coefficient diagram showing the relation between phenotypic measurements (X_i) and the aggregate genotype (H). For further explanation of symbols see text.

Let an observed trait (X) be the sum of the average effects of the genes (G)[3] which an animal inherits, plus the combined effects of environment, dominance and epistasis (E); thus

$$X = G + E. \tag{7}$$

Then the observed variance is

$$\sigma_X^2 = \sigma_G^2 + \sigma_E^2, \tag{8}$$

[3] We have defined the genotype as the sum of the average effects of genes because WRIGHT (1921, 1935) has shown that only the average (additive) effects contribute to permanent gain from selection. The effects of environment, dominance, and epistasis may logically be grouped together since they act similarly to mask the genotype.

if G and E are uncorrelated, as would be the case unless particular efforts were made to give the better genotypes better or worse than average treatment. The correlation between the genotype and phenotype for the same trait is

$$r_{G_iX_i} = \frac{\sigma_{G_i}}{\sigma_{X_i}} = g_i, \tag{9}$$

where that trait is measured on the animal itself. The term g_i is the square root of the heritable (additively genetic) fraction of the observed variance.

DARWIN observed the importance of correlated variation, as evidenced by the statement, "Hence if man goes on selecting, and thus augmenting, any peculiarity, he will almost certainly modify unintentionally other parts of the structure, owing to the mysterious laws of correlation." The genetic basis for this statement is evident if genes have manifold effects. Many cases of pleiotropic genes have been reported for laboratory animals, although they have received little attention in domestic animals. Linkage and non-random mating systems may also cause correlated variation; however, their effects would be less permanent and consequently less important in selection. Repeated crossing over ultimately makes the coupling and repulsion heterozygotes equally numerous, while the transient nature of breeding herds prevents sustained departure from random mating in most cases. An additional and usually much more important cause of correlated variation within an interbreeding population lies in the environmental circumstances peculiar to each animal, particularly for traits which develop during the same, or in adjacent, periods of time. Thus if trait I is correlated with trait J in the same animal, I may serve as an indicator either of the animal's genotype for J or of the environmental circumstances to which that animal was exposed when traits I and J were both being developed.

Statistically an observed phenotypic correlation may be analyzed into its constituents, a genetic correlation ($r_{G_iG_j}$) and an environmental correlation ($r_{E_iE_j}$), as indicated in figure 1. The observed phenotypic correlation between two traits measured on the same animal is

$$r_{X_iX_j} = g_ig_jr_{G_iG_j} + e_ie_jr_{E_iE_j}, \quad \text{where} \quad e_i = \frac{\sigma_{E_i}}{\sigma_{X_i}}. \tag{10}$$

Sex-limited traits and traits such as carcass merit cannot be measured directly on all breeding animals. Sometimes selection must be practiced before each animal's performance for every trait is known. For these reasons there is a possibility of increasing genetic progress in a breeding program by using information about the performance of relatives. Examples of how r_{X_iH} may be calculated are given in the subsequent section where X_i is (1) the individual's own performance, (2) the performance of the individual's dam, and (3) the average perfromance of a group of relatives of which the individual is a member.

APPLICATION

Three selection indexes for young boars and gilts were constructed from data taken on the Iowa Station swine herd from the fall of 1937 through the spring of 1940. The history of the herd was given by BYWATERS (1937) and more recently by WHATLEY (1942). LUSH (1940) showed that the economic return from the swine enterprise depended largely upon three traits—growth rate, market suitability, and size of litter—the relative economic values of which were ½, 1, and 2, respectively, in the units used in this study. Growth rate was measured by weight at 180 days of age. Market suitability was measured by a numerical score given by each of several judges. The details of these measurements were given by WHATLEY for 180-day weight and by STONAKER and LUSH (1942) for score. The measure of litter size was that suggested by LUSH and MOLLN (1942),

$$P = n_0 + n_{21} + n_{56} + W_{21}/10 + W_{56}/30,$$

the n's and W's referring to the number of pigs and weight of the litter, respectively, at the ages designated by the subscripts. Young boars and gilts are not old enough to have produced litters themselves at the time first selections must be made; consequently the estimate of productivity for each young animal is based upon the litter in which it was born.

The aggregate breeding value of an animal is therefore defined as

$$H = \tfrac{1}{2}G_W + G_S + 2G_P,$$

G_W, G_S and G_P referring to the genotypes for 180-day weight, market score and productivity, respectively. The following statistics are needed to construct one or more of the three indexes:

A. Phenotypic constants
   1. Standard deviation for each of the three traits
   2. Phenotypic correlation between each pair of traits
   3. Phenotypic correlations between the traits of relatives

B. Genetic constants
   1. Heritable fraction of the variance in each trait
   2. Genetic correlation between each pair of traits.

Differences due to season and line of breeding were eliminated in calculating these statistics, as selection is usually practiced between animals born in the same season and within the same line or other interbreeding population. The phenotypic constants shown in table 1 were calculated by the usual methods of variance and covariance analysis (SNEDECOR 1940).

Several procedures were outlined by LUSH (1941) for estimating heritability (g_1^2) in farm livestock. Intrasire regressions of offspring on dam were considered preferable to correlation coefficients in the present case because of the rather intense selection which had been practiced in the Iowa Station swine

TABLE 1

Phenotypic constants necessary to construct one or more of the three indexes.

TRAIT	STANDARD DEVIATION	DEGREES OF FREEDOM
180-day weight (in pounds)	31.86	1513
Market score (in points)	4.78	1513
Productivity (in points)	9.72	282
CORRELATION BETWEEN TRAITS	**CORRELATION COEFFICIENT**	**STANDARD ERROR**
Pig's own weight and score	0.614	0.016
Pig's own weight and productivity of its dam	−.024	.059
Pig's own score and productivity of its dam	−.081	.059
Weight of litter mates	.314	.027
Score of litter mates	.204	.026
Weight and score of litter mates	.186	.026

herd for the traits under consideration. The intensity of selection was evidenced by the higher averages and lower variability for the dams as compared to their offspring. The intrasire regressions of offspring on dam are shown in table 2.

TABLE 2

Regression of each trait for the offspring on each of the three traits for the dam.

OFFSPRING'S TRAIT	DAM'S TRAIT	SYMBOL*	REGRESSION COEFFICIENT	DEGREES OF FREEDOM
Weight	Weight	$b_{W_2W_1}$	0.149	232
Score	Score	$b_{S_2S_1}$	.049	189
Productivity	Productivity	$b_{P_2P_1}$	.081	82
Weight	Score	$b_{W_2S_1}$	.153	189
Score	Weight	$b_{S_2W_1}$	.013	232
Weight	Productivity	$b_{W_2P_1}$	−.067	204
Productivity	Weight	$b_{P_2W_1}$	.004	52
Score	Productivity	$b_{S_2P_1}$	−.024	204
Productivity	Score	$b_{P_2S_1}$	.041	52

* The sub-subscripts 1 and 2 refer to dam and offspring, respectively, the regressions being those of offspring's trait on dam's trait in each case.

Values of g_1, calculated from the formula,[4] $g_1=\sqrt{2b_{i_2i_1}}$, are given in table 3 for the three traits.

[4] This formula was derived from the relation,

$$b_{i_2i_1}=g_1^2t+e_1^2r_{EE}.$$

The genetic relationship between dam and offspring (t) was taken as 0.5, and the environmental correlation (r_{EE}) was assumed zero, since no particular efforts had been made to give parent and offspring similar or dissimilar treatment.

TABLE 3

Genetic constants derived from intrasire regressions of offspring on dam.

STATISTIC	SYMBOL	ESTIMATE
$\sqrt{\text{heritability of weight}}$	g_W	0.546
$\sqrt{\text{heritability of score}}$	g_S	.313
$\sqrt{\text{heritability of productivity}}$	g_P	.402
Genetic correlation between weight and score	$r_{G_WG_S}$	.519
Genetic correlation between weight and productivity	$r_{G_WG_P}$	.0±
Genetic correlation between score and productivity	$r_{G_SG_P}$	.0±

Procedures for estimating the genetic correlations ($r_{G_iG_j}$) have not been developed previously. Equation 10 is not suitable for this purpose because no direct method is available for separating the genetic and environmental correlations for two traits measured upon the same animal. For example, any environmental accident such as differential exposure to parasites or infection which affected growth and plumpness would either raise or lower the growth rate and score of the same animal. Thus the actually observed correlation between the two traits on the same animal conceivably could be due wholly to such environmental circumstances, or to genes which affect both traits, or to a mixture of the two causes in any proportion. To measure the genetic correlations by themselves it was necessary to correlate one trait in one animal with the other in a relative. The formula,[5]

$$r_{G_iG_j} = \sqrt{\frac{b_{i_2i_1} \cdot b_{j_2j_1}}{b_{i_2i_1} \cdot b_{j_2j_1}}} = \sqrt{\frac{(\text{cov } I_2J_1)(\text{cov } J_2I_1)}{(\text{cov } I_2I_1)(\text{cov } J_2J_1)}},$$

was adopted because it appeared to be unbiased by selection and to utilize most of the available information. Estimates of the genetic correlations between each pair of the three traits are given in table 3. Consistent estimates could not be derived for $r_{G_WG_P}$ and $r_{G_SG_P}$, since in each case the two regressions required in the numerator differed in sign. Since these regressions are small and not significantly different from zero, it seemed more accurate to assign both $r_{G_WG_P}$ and $r_{G_SG_P}$ values of zero than to attempt an alternative method of calculating them.

The genetic constants probably include a small fraction of the epistatic deviations and exclude the average differences between groups of dams to

[5] Selection for the independent variable does not bias the corresponding regression coefficient (EISENHART 1939). The quantity $\sqrt{b_{i_2i_1} \cdot b_{j_2j_1}}$ consequently can be taken as an estimate of the correlation between two traits of dam and offspring in an unselected population. The biometric relation,

$$r_{i_2i_1} = r_{j_2i_1} = g_i g_j t r_{G_iG_j},$$

permits the derivation of the formula given above for $r_{G_iG_j}$. The two regressions (or correlations) actually provide two independent estimates of $r_{G_iG_j}$ in unselected populations and an arithmetic average of the two estimates would be less biased by sampling errors than the geometric average used here.

which sires were mated. Since some inbreeding had occurred, they may be slightly nearer zero than would be expected in a non-inbred population. If they are biased, and this bias is equal and in the same direction for the different genetic constants, the index will be no less accurate for selecting breeding animals.

Weight at 180 days of age and market score are available for each animal before breeding age. The first index constructed was based on these two traits. Values for r_{WH} and r_{SH} were calculated by substituting the statistics from tables 1 and 3 into formula 6 as follows:

$$r_{WH} = \frac{0.546}{\sigma_H}\{5.803(1) + (1.496)(.519)\} = 3.593/\sigma_H$$

$$r_{SH} = \frac{0.313}{\sigma_H}\{5.803(.519) + 1.496(1)\} = 1.409/\sigma_H.$$

The two simultaneous equations are:

$$\beta_W + 0.614\beta_S = 3.593/\sigma_H$$
$$0.614\beta_W + \beta_S = 1.409/\sigma_H.$$

Solving, we get $\beta_W = 4.381/\sigma_H$ and $\beta_S = -1.282/\sigma_H$.
Since $\beta_i = b_i\sigma_{Xi}/\sigma_H$, the first index is

$$I_1 = 0.137W - 0.268S,$$

where W and S represent the pig's own 180-day weight and market score, respectively. If the index can thus be made more convenient for use or for keeping records, it may be multiplied by any constant or any constant may be added to it without influencing its relative accuracy, since such procedure does not change R_{IH}.

A second index was constructed by using the productivity of the dam as a measure of each pig's productivity in the index, the lapse of one generation being compensated by multiplying r_{PH} by one-half as follows:

$$r_{PH} = \frac{0.402}{2\sigma_H}(7.802) = 1.566/\sigma_H.$$

The three simultaneous equations were solved as before, the second index being

$$I_2 = 0.136W - 0.232S + 0.164P.$$

The third index was designed to include information about the average weight and score of the litter in which each pig was born, in addition to the three traits in the second index. The procedure used was to consider the average weight ($\overline{W}$) and score ($\overline{S}$) of the litter as a fourth and fifth variable, expressing the necessary correlations in terms of the correlations given in table 1 and as a function of the number of pigs per litter (k). This latter step was necessary because the number upon which the average is based influences the

variability of the average and the amount of information in the average concerning each pig's breeding value. The additional phenotypic correlations in terms of k and the correlations in table 1 are

$$r_{w\bar{w}} = \sqrt{\frac{1 + (k-1).314}{k}},$$

$$r_{s\bar{s}} = \sqrt{\frac{1 + (k-1).204}{k}},$$

$$r_{w\bar{s}} = \frac{.614 + (k-1).186}{\sqrt{k[1 + (k-1).204]}},$$

$$r_{s\bar{w}} = \frac{.614 + (k-1).186}{\sqrt{k[1 + (k-1).314]}},$$

$$r_{p\bar{w}} = -.024\sqrt{\frac{k}{1 + (k-1).314}}$$

$$r_{p\bar{s}} = -.081\sqrt{\frac{k}{1 + (k-1).204}}, \quad \text{and}$$

$$r_{\bar{w}\bar{s}} = \frac{.614 + (k-1).186}{\sqrt{[1 + (k-1).314][1 + (k-1).204]}}.$$

The additional correlations between H and the litter averages are

$$r_{\bar{w}H} = \frac{3.593[1 + (k-1).5]}{\sigma_H\sqrt{k[1 + (k-1).314]}} \quad \text{and}$$

$$r_{\bar{s}H} = \frac{1.409[1 + (k-1).5]}{\sigma_H\sqrt{k[1 + (k-1).204]}}.$$

The five simultaneous equations were solved as before, giving the following partial regression coefficients:

$$b_w = .098$$

$$b_s = -.165$$

$$b_p = \frac{0.358\{0.164(k-1) + 0.016(k-1)^2\}}{2.18\{0.998(k-1) + 0.899(k-1)^2\}}$$

$$b_{\bar{w}} = \frac{k\{0.270 + (0.004)(k-1)\}}{7.14\{3.273(k-1) + 0.324(k-1)^2\}}$$

$$b_{\bar{s}} = \frac{-k\{0.070 + (0.02)(k-1)\}}{1.07\{0.491(k-1) + 0.049(k-1)^2\}}.$$

The absolute values of the three latter coefficients are given in table 4 for different values of k. The number of pigs in the litter has so little effect on

TABLE 4

Partial regression coefficients for dam's productivity, average weight of the litter and average score of the litter for different numbers of pigs in the litter, in the third index.

NUMBER OF PIGS IN THE LITTER (k)	b_P	$b_{\bar{W}}$	$b_{\bar{S}}$
1	0.164	0.038	−0.067
2	.165	.059	− .113
3	.165	.072	− .148
4	.166	.081	− .175
5	.166	.088	− .197
6	.167	.093	− .215
7	.167	.098	− .230
8	.167	.101	− .243
9	.168	.104	− .254
10	.168	.106	− .264
11	.168	.109	− .273
12	.169	.110	− .280
13	.169	.112	− .287
14	.169	.113	− .293
15	.169	.115	− .299

b_P that this could be taken as a constant (0.166 or 0.167) with very little error.

The amount of genetic progress expected when a given index is used in making selections is proportional to R_{IH} (see formula 3). Hence these values provide a basis for choosing an index which is easy and simple to use yet which is of nearly maximum accuracy. For example the three indexes previously constructed may be compared as follows:

$$R_{I_1H} = \sqrt{\beta_W r_{WH} + \beta_S r_{SH}} = 0.363$$

$$R_{I_2H} = 0.395 \quad \text{and}$$

$$R_{I_3H} = 0.404, \quad \text{for} \quad k = 5.$$

The second and third indexes are 8.8 and 11.3 percent, respectively, more efficient than the first. Since the time and effort expended in keeping records is but a small fraction of the total labor connected with a breeding program, the second index would almost certainly be preferable to the first. The third might also be chosen over the second, since genetic progress could be increased still further, and the extra labor would be only that of computing and using the litter averages from data already taken.

DISCUSSION

FISHER'S (1930) "fundamental theorem of natural selection" and WRIGHT'S (1931) emphasis that genetic change depends upon genetic variability and selection intensity indicate that the factors which are important in natural selection also hold for selection as practiced by man. In equation 3, σ_H is a

measure of genetic variability and $\bar{\imath}$ a measure of selection intensity. The breeder in applying artificial selection to an animal population has the opportunity of increasing the accuracy of his selections (increasing R_{IH}) considerably over what may hold in the "trial-and-error" methods of natural selection. The breeder has additional opportunities of increasing $\bar{\imath}$ (within limits) and of increasing genetic variability by the intentional control of population size (inbreeding) and migration (outcrossing) which have not been considered in the present study. WRIGHT (1940) has examined the conditions under which the supplementary use of these latter methods may be advantageous.

For the special case where the traits are uncorrelated, R_{IH} is a maximum when each regression coefficient is equal to (or proportional to) the product of the relative economic value and heritability for each trait ($b_i = a_i g_i^2$). The correlations which may exist between traits complicate the calculation of the partial regression coefficients, just as correlations between the independent variables do in any multiple regression analysis. When the phenotypic correlation is large as compared to the genetic correlation, the regression coefficient for a trait with little economic importance or slight heritable variation may be negative, because its function in the index then becomes mainly that of indicating the environment for a more important and more highly heritable trait. An example of this was seen in the negative regression coefficients for score in the three indexes calculated previously. However, selection for the animals having the highest indexes would create some improvement for score because of the positive genetic correlation between score and weight.

An index constructed from data taken on a herd in one locality may not be widely applicable. The reasons for this are:

1. Relative economic values for a trait may vary with the particular locality or nature of the enterprise.

2. The genetic constitution of herds may differ, especially where they are under distinctly non-random mating systems such as intense inbreeding.

3. Different managerial practices may cause the standard deviations for the traits to vary in different herds. The standard deviations for subjective traits such as market conformation measured by judging or by scores may vary because different judges will vary the range over which they spread their scores.

4. Few herds are large enough to provide data sufficient to make the sampling errors of the genetic constants small.

The best way to test whether or not selection indexes can be standardized and recommended for general use seems to be to compare several indexes constructed from data taken on different herds.

The data in the present study were sufficiently numerous to provide accurate estimates of the phenotypic constants in table 1. They were less satisfactory with regard to the reliability of the genetic constants given in table 3. Some idea of the general accuracy of these figures can be obtained by comparing them to similar estimates by other investigators. WHATLEY (1942) used several methods to estimate heritability of 180-day weight in the Iowa Station swine herd through 1938, concluding that "at least 30 percent and possibly

more than 40 percent of the individual variance" was hereditary. WHATLEY and NELSON (1942) estimated that 180-day weight in the Oklahoma Duroc herd was 23 percent heritable, while BAKER and coworkers (1943) found a figure of 25 percent for 168-day weight in the Nebraska (North Platte substation) Duroc herd. These estimates help to substantiate the figure of 30 percent for the heritability of 180-day weight found in this study. WHATLEY and NELSON also estimated that market score was about 33 percent heritable, while STONAKER and LUSH (1942) obtained an estimate of 20 percent for the Iowa Station herd from data which included that used in this study. These estimates indicate that our estimate of 10 percent for the heritability of score may be too small. Previous reports of the heritability of productivity have not been made, but LUSH and MOLLN (1942) found that the correlation between litters by the same sow was between 0.15 and 0.20 for the items which are included in productivity. They quoted a number of investigations which substantiated their findings. In general these results substantiate our figure of 16 percent if most of the permanent differences in these items between sows are hereditary.

Other attempts to estimate genetic correlations have not been made. The genetic correlation of 0.52 between 180-day weight and score indicates that about half of the genes which influence one trait also influence the other (assuming equal gene frequency and equal effects of the genes). However most of the observed correlation of 0.61 was due to an environmental correlation. Although an effort was made to score all pigs at a constant weight of 225 pounds, part of this correlation was probably due to a subjective tendency on the part of the judges to assign scores in accordance with differences in age or weights of the pigs. The genetic correlations between productivity and weight or score may have been either positive or negative so far as the evidence from the present data indicate. They appeared to be small; hence assigning them a value of zero is unlikely to have caused serious errors in the indexes.

From the studies of heritability which have been made for economic traits in different farm animals, it seems that the best indexes which can be constructed will be far from perfect. The confusing effects of environment, dominance, and epistasis in masking genotypes cause the progress in the present case to be less than half of what might be made if genotypes could be recognized precisely. Thus the indexes constructed for swine permit from 36.1 to 40.4 percent as much gain as could be made with a perfect index (where $R_{IH} = 1$), which is the limit of what could be achieved if the exact Mendelian composition of every animal were known. These indexes could be improved somewhat by more perfect control of the environment, by the wise use of corrections for known environmental circumstances, by more accurately measuring differences in phenotypes, and by including the performance of additional relatives in the index; however, the use of these methods is limited by practical considerations. Although R_{IH} is likely to increase with the age of the animal (as more becomes known about its phenotype and as its progeny becomes observable) so that more gain can be made from selecting within a group of

older animals, the length of generation will also increase. DICKERSON and HAZEL (1942) have shown that the interval between generations in some cases is increased by progeny testing more than enough to offset the increased accuracy of selection, the net result of more emphasis on the progeny test then being a decrease in the *annual* rate of genetic improvement. While these considerations do not indicate much possibility of phenomenally rapid improvement in animal populations from selection alone, the progress which can be made with properly constructed indexes is considerably greater than can be expected when the ideals toward which selection is directed are confused or erroneous.

CONCLUSIONS

The genetic gain which can be made by selecting for several traits simultaneously within a group of animals is the product of (1) the selection differential, (2) the multiple correlation between aggregate breeding value and the selection index, and (3) genetic variability. The first of these may be very small due to the breeder's carelessness, procrastination, etc., and is limited by the rate of reproduction for each species, while the third is relatively beyond man's control; hence the greatest opportunity of increasing the progress from selection is by insuring that the second is as large as possible.

A multiple correlation method of constructing selection indexes having maximum accuracy was presented. The following constants must be known in order to solve the simultaneous equations:

1. Relative economic values for the different traits
2. Phenotypic constants
   a. Standard deviations for each trait
   b. Correlation between each pair of traits
3. Genetic constants
   a. Heritability of each trait
   b. Genetic correlations between each pair of traits

Examples of the construction of selection indexes for young boars and gilts were presented from data taken on the Iowa Station swine herd using (1) 180-day weight and market score of the individual animal, (2) the two previous traits and productivity of the dam, and (3) the three previous traits and the average weight and score of the litter of which each pig is a member.

The progress which can be made by using the above indexes varied from 36 to 40 percent of that which could be made with a perfect index. The loss is due to the confusing effects of environmental circumstances, dominance, and epistasis, all of which can make phenotypes unlike genotypes.

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MUTATIONS OF BACTERIA FROM VIRUS SENSITIVITY TO VIRUS RESISTANCE[1,2]

S. E. LURIA[3] AND M. DELBRÜCK
Indiana University, Bloomington, Indiana, and
Vanderbilt University, Nashville, Tennessee

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INTRODUCTION

WHEN a pure bacterial culture is attacked by a bacterial virus, the culture will clear after a few hours due to destruction of the sensitive cells by the virus. However, after further incubation for a few hours, or sometimes days, the culture will often become turbid again, due to the growth of a bacterial variant which is resistant to the action of the virus. This variant can be isolated and freed from the virus and will in many cases retain its resistance to the action of the virus even if subcultured through many generations in the absence of the virus. While the sensitive strain adsorbed the virus readily, the resistant variant will generally not show any affinity to it.

The resistant bacterial variants appear readily in cultures grown from a single cell. They were, therefore, certainly not present when the culture was started. Their resistance is generally rather specific. It does not extend to viruses that are found to differ by other criteria from the strain in whose presence the resistant culture developed. The variant may differ from the original strain in morphological or metabolic characteristics, or in serological type or in colony type. Most often, however, no such correlated changes are apparent, and the variant may be distinguished from the original strain only by its resistance to the inciting strain of virus.

The nature of these variants and the manner in which they originate have been discussed by many authors, and numerous attempts have been made to correlate the phenomenon with other instances of bacterial variation.

The net effect of the addition of virus consists of the appearance of a variant strain, characterized by a new stable character—namely, resistance to the inciting virus. The situation has often been expressed by saying that bacterial viruses are powerful "dissociating agents." While this expression summarizes adequately the net effect, it must not be taken to imply anything about the mechanism by which the result is brought about. A moment's reflection will show that there are greatly differing mechanisms which might produce the same end result.

D'HERELLE (1926) and many other investigators believed that the virus by direct action induced the resistant variants. GRATIA (1921), BURNET (1929), and others, on the other hand, believed that the resistant bacterial variants are produced by mutation in the culture prior to the addition of virus. The

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[3] Fellow of the GUGGENHEIM FOUNDATION.

virus merely brings the variants into prominence by eliminating all sensitive bacteria.

Neither of these views seems to have been rigorously proved in any single instance. BURNET'S (1929) work on isolations of colonies, morphologically distinguishable prior to the addition of virus, which proved resistant to the virus comes nearest to this goal. His results appear to support the mutation hypothesis for colony variants. It may seem peculiar that this simple and important question should not have been settled long ago, but a close analysis of the problem in hand will show that a decision can only be reached by a more subtle quantitative study than has hitherto been applied in this field of research.

Let us begin by restating the basic experimental finding.

A bacterial culture is grown from a single cell. At a certain moment the culture is plated with virus in excess. Upon incubation, one finds that a very small fraction of the bacteria survived the attack of the virus, as indicated by the development of a small number of resistant colonies, consisting of bacteria which do not even adsorb the virus.

Let us focus our attention on the first generation of the resistant variant—that is, on those bacteria which survive immediately after the virus has been added. These survivors we may call the "original variants." We know that these bacteria and their offspring are resistant to the virus. We may formulate three alternative hypotheses regarding them.

a. *Hypothesis of mutation to immunity.* The original variants were resistant before the virus was added, and, like their offspring, did not even adsorb it. On this hypothesis the virus did not interact at all with the original variants, the origin of which must be ascribed to "mutations" that occur quite independently of the virus. Naming such hereditary changes "mutations" of course does not imply a detailed similarity with any of the classes of mutations that have been analyzed in terms of genes for higher organisms. The similarity may be merely a formal one.

b. *Hypothesis of acquired immunity.* The original variants interacted with the virus, but survived the attack. We may then inquire into the predisposing cause which effected the survival of these bacteria in contradistinction to the succumbing ones. The predisposing cause may be hereditary or random. Accordingly we arrive at two alternative hypotheses—namely,

b_1. *Hypothesis of acquired immunity of hereditarily predisposed individuals.* The original variants originated by mutations occurring independently of the presence of virus. When the virus is added, the variants will interact with it, but they will survive the interaction, just as there may be families which are hereditarily predisposed to survive an otherwise fatal virus infection. Since we know that the offspring of the original variants do not adsorb the virus, we must further assume that the infection caused this additional hereditary change.

b_2. *Hypothesis of acquired immunity—hereditary after infection.* The original variants are predisposed to survival by random physiological variations in size, age, etc. of the bacteria, or maybe even by random variations in the

point of attack of the virus on the bacterium. After survival of such random individuals, however, we must assume that their offspring are hereditarily immune, since they do not even adsorb the virus.

These alternative hypotheses may be grouped by first considering the origin of the hereditary difference. Do the original variants trace back to mutations which occur independently of the virus, such that these bacteria belong to a few clones, or do they represent a random sample of the entire bacterial population? The first alternative may then be subdivided further, according to whether the original variants do or do not interact with the virus. Disregarding for the moment this subdivision, we may formulate two hypotheses:

1. *First hypothesis* (*mutation*): There is a finite probability for any bacterium to mutate during its life time from "sensitive" to "resistant." Every offspring of such a mutant will be resistant, unless reverse mutation occurs. The term "resistant" means here that the bacterium will not be killed if exposed to virus, and the possibility of its interaction with virus is left open.

2. *Second hypothesis* (*acquired hereditary immunity*): There is a small finite probability for any bacterium to survive an attack by the virus. Survival of an infection confers immunity not only to the individual but also to its offspring. The probability of survival in the first instance does not run in clones. If we find that a bacterium survives an attack, we cannot from this information infer that close relatives of it, other than descendants, are likely to survive the attack.

The last statement contains the essential difference between the two hypotheses. On the mutation hypothesis, the mutation to resistance may occur any time prior to the addition of virus. The culture therefore will contain "clones of resistant bacteria" of various sizes, whereas on the hypothesis of acquired immunity the bacteria which survive an attack by the virus will be a random sample of the culture.

For the discussion of the experimental possibility of distinction between these two hypotheses, it is important to keep in mind that the offspring of a *tested* bacterium which survives is resistant on either hypothesis. Repeated tests on a bacterium at different times, or on a bacterium and on its offspring, could therefore give no information of help in deciding the present issue. Thus, one has to resort to less direct methods. Two main differences may be derived from the hypotheses:

First, if the individual cells of a very large number of microcolonies, each containing only a few bacteria, were examined for resistance, a pronounced correlation between the types found in a single colony would be expected on the mutation hypothesis, while a random distribution of resistants would be expected on the hypothesis of acquired hereditary immunity. This experiment, however, is not practicable, both on account of the difficulty of manipulation and on account of the small proportion of resistant bacteria.

Second, on the hypothesis of resistance due to mutation, the proportion of resistant bacteria should increase with time, in a growing culture, as new mutants constantly add to their ranks.

In contrast to this increase in the proportion of resistants on the mutation hypothesis, a constant proportion of resistants may be expected on the hypothesis of acquired hereditary immunity, as long as the physiological conditions of the culture do not change. To test this point, accurate determinations of the proportion of resistant bacteria in a growing culture and in successive subcultures are required. In the attempt to determine accurately the proportion of resistant bacteria, great variations of the proportions were found, and results did not seem to be reproducible from day to day.

Eventually, it was realized that these fluctuations were not due to any uncontrolled conditions of our experiments, but that, on the contrary, large fluctuations are a necessary consequence of the mutation hypothesis and that the quantitative study of the fluctuations may serve to test the hypothesis.

The present paper will be concerned with the theoretical analysis of the probability distribution of the number of resistant bacteria to be expected on either hypothesis and with experiments from which this distribution may be inferred.

While the theory is here applied to a very special case, it will be apparent that the problem is a general one, encountered in any case of mutation in uniparental populations. It is the belief of the authors that the quantitative study of bacterial variation, which until now has made such little progress, has been hampered by the apparent lack of reproducibility of results, which, as we shall show, lies in the very nature of the problem and is an essential element for its analysis. It is our hope that this study may encourage the resumption of quantitative work on other problems of bacterial variation.

THEORY

The aim of the theory is the analysis of the probability distributions of the number of resistant bacteria to be expected on the hypothesis of acquired immunity and on the hypothesis of mutation.

The basic assumption of the hypothesis of acquired hereditary immunity is the assumption of a fixed small chance for each bacterium to survive an attack by the virus. In this case we may therefore expect a binomial distribution of the number of resistant bacteria, or, in cases where the chance of survival is small, a Poisson distribution.

The basic assumption of the mutation hypothesis is the assumption of a fixed small chance *per time unit* for each bacterium to undergo a mutation to resistance. The assumption of a fixed chance per time unit is reasonable only for bacteria in an identical state. Actually the chance may vary in some manner during the life cycle of each bacterium and may also vary when the physiological conditions of the culture vary, particularly when growth slows down on account of crowding of the culture. With regard to the first of these variations, the assumed chance represents the average chance per time unit, averaged over the life cycle of a bacterium. With regard to the second variation, it seems reasonable to assume that the chance is proportional to the growth rate of the bacteria. We will then obtain the same results as on the simple assump-

tion of a fixed chance per time unit, *if we agree to measure time in units of division cycles of the bacteria*, or any proportional unit.

We shall choose as time unit the average division time of the bacteria, divided by ln 2, so that the number N_t of bacteria in a growing culture as function of time t follows the equations

$$(1) \qquad dN_t/dt = N_t, \quad \text{and} \quad N_t = N_0 e^t.$$

We may then define the chance of mutation for each bacterium during the time element dt as

$$(2) \qquad adt,$$

so that a is the chance of mutation per bacterium per time unit, or the "mutation rate."

If a bacterium is capable of different mutations, each of which results in resistance, the mutation rate here considered will be the sum of the mutation rates associated with each of the different mutations.

The number dm of mutations which occur in a growing culture during a time interval dt is then equal to this chance (2) multiplied by the number of bacteria,[4] or

$$(3) \qquad dm = adtN_t,$$

and from this equation the number m of mutations which occur during any finite time interval may be found by integration to be

$$(4) \qquad m = a(N_t - N_0)$$

or, in words, to be equal to the chance of mutation per bacterium per time unit multiplied by the increase in the number of bacteria.

The bacteria which mutate during any time element dt form a random sample of the bacteria present at that time. For small mutation rates, their number will therefore be distributed according to Poisson's law. Since the mutations occuring in different time intervals are quite independent from each other, the distribution of all mutations will also be according to Poisson's law.

This prediction cannot be verified directly, because what we observe, when we count the number of resistant bacteria in a culture, is not the number of mutations which have occurred, but the number of resistant bacteria which have arisen by multiplication of those which mutated, the amount of multiplication depending on how far back the mutation occurred.

If, however, the premise of the mutation hypothesis can be proved by other means, the prediction of a Poisson distribution of the number of mutations

[4] We assume that the number of resistant bacteria is at all times small in comparison with the total number of bacteria. If this condition is not fulfilled, the total number of bacteria in this equation has to be replaced by the number of sensitive bacteria. The subsequent theoretical developments will then become a little more complicated. For the case studied in the experimental part of this paper the condition is fulfilled.

may be used to determine the mutation rate. It is only necessary to determine the fraction of cultures showing no mutation in a large series of similar cultures. This fraction p_0, according to theory, should be:

$$p_0 = e^{-m}. \tag{5}$$

From this equation the average number m of mutations may be calculated, and hence the mutation rate a from equation (4).

Let us now turn to the discussion of the distribution of the number of resistant bacteria.

The average number of resistant bacteria is easily obtained by noting that this number increases on two accounts—namely, first on account of new mutations, second on account of the growth of resistant bacteria from previous mutations. During a time element dt the increase on the first account will be, by equation (3): $adtN_t$. N_t, the number of bacteria present at time t, is given by equation (1). The increase on the second account will depend on the growth rate of the resistant bacteria. In the simple case, which we shall treat here, this growth rate is the same as that of the sensitive bacteria, and the increment on this account is $\rho\, dt$, where ρ is the average number of resistant bacteria present at time t. We have then as the total rate of increase of the average number of resistant bacteria $d\rho/dt = aN_t + \rho$ and upon integration

$$\rho = taN_t \tag{6}$$

if we assume that at time zero the culture contained no resistant bacteria.

It will be seen that the average number of resistant bacteria increases more rapidly than the total number of bacteria. Indeed the fraction of resistant bacteria in the culture increases proportionally to time. This, as pointed out in the introduction, is a distinguishing feature of the mutation hypothesis but unfortunately, as will be seen in the sequel, is not susceptible to experimental verification due to statistical fluctuations.

The resistant bacteria in any culture may be grouped, for the purpose of this analysis, into clones, taking together all those which derive from the same mutation. We may say that the culture contains clones of various age and size, calling "age" of a clone the time since its parent mutation occurred and "size" of a clone the number of bacteria in a clone at the time of observation. It is clear that size and age of a clone determine each other. If, in particular, we make the simplifying hypothesis that the resistant bacteria grow as fast as the normal sensitive strain, the relation between size and age will be expressed by equation (1), with appropriate meaning given to the symbols.

The relation implies that the size of a clone increases exponentially with its age. On the other hand, the frequency with which clones of different ages may be encountered in any culture must decrease exponentially with age, according to equations (3) and (1).

Combining these two results—namely, that clone size increases exponentially with clone age and that frequency of clones of different age decreases exponentially with clone age—we see that the two factors cancel when the

average number of bacteria belonging to clones of one age group is considered. In other words, at the time of observation we shall have, *on the average*, as many resistant bacteria stemming from mutations which occurred during the first generation after the culture was started as stemming from mutations which occurred during the last generation before observation, or during any other single generation.

On the other hand, for small mutation rates it is very improbable that any mutation will occur during the early generations of a single or of a limited number of experimental cultures. It follows that the average number of resistant bacteria derived from a limited number of experimental cultures will, probably, be considerably smaller than the theoretical value given by equation (6), and, improbably, the experimental value will be much larger than the theoretical value. The situation is similar to the operation of a (fair) slot machine, where the average return from a limited number of plays is probably considerably less than the input, and improbably, when the jackpot is hit, the return is much bigger than the input.

This result characterizes the distribution of the number of resistant bacteria as a distribution with a long and significant tail of rare cases of high numbers of resistant bacteria, and therefore as *a distribution with an abnormally high variance*. This variance will be calculated below.

For such distributions the averages derived from limited numbers of samples yield very poor estimates of the true averages. Somewhat better estimates of the averages may in such cases be obtained by omitting, in the calculation of the theoretical averages, the contribution to these averages of those events which probably will not occur in any of our limited number of samples. We may do this, in the integration leading to equation (6), by putting the lower limit of integration not at time zero, when the cultures were started, but at a certain time t_0, prior to which mutations were not likely to occur in any of our experimental cultures. We then obtain as a *likely average* r of the number of resistant bacteria in a limited number of samples, instead of equation (6),

$$r = (t - t_0)aN_t. \tag{6a}$$

It now remains to choose an appropriate value for the time interval $t - t_0$.

For this purpose we return to equation (4), in which it was stated that the average number of mutations which occur in a culture is equal to the mutation rate multiplied by the increase of the number of bacteria. Let us then choose t_0 such that up to that time just one mutation occurred, on the average, in a group of C similar cultures, or

$$1 = aC(N_{t_0} - N_0).$$

In this equation we may neglect N_0, the number of bacteria in each inoculum, in comparison with N_{t_0}, the number of bacteria in each culture at the critical time t_0. We may also express N_{t_0} in terms of N_t, the number of bacteria at the time of observation, applying equation (1):

$$N_{t_0} = N_t e^{-(t-t_0)}.$$

We thus obtain

$$t - t_0 = \ln(N_t C a). \tag{7}$$

Equations (6a) and (7) may be combined to eliminate $t - t_0$ and to yield a relation between the observable quantities r and N_t on the one hand and the mutation rate a on the other hand, to be determined by this equation:

$$r = aN_t \ln (N_t C a). \tag{8}$$

This simple transcendental equation determining a may be solved by any standard numerical method. In figure 1, the relation between r and aN_t is plotted for several values of C.

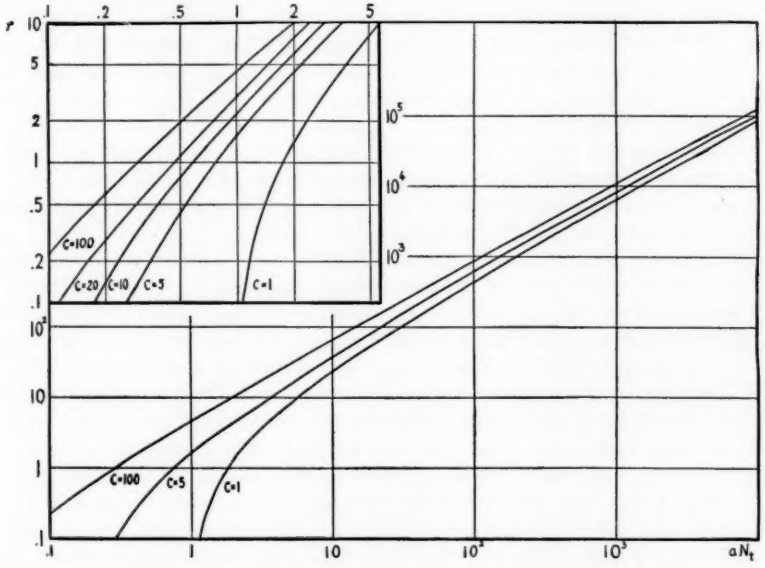

FIGURE 1.—The value of aN_t as a function of r for various values of C. The upper left hand part of the figure gives the curves for low values of aN_t and of r on a larger scale. See text.

Estimates of a obtained from equation (8) will be too high if in any of the experimental cultures a mutation happened to occur prior to time t_0. From the definition of t_0 it will be seen that this can be expected to happen in little more than half of the cases.

While we have thus obtained a relation permitting an estimate of the mutation rate from the observation of a limited number of cultures, this relation is in no way a test of the correctness of the underlying assumptions and, in particular, is not a test of the mutation hypothesis itself. In order to find such tests of the correctness of the assumption we must derive further quantitative relations concerning the distribution of the number of resistant bacteria and compare them with experimental results.

Since we have seen that the mutation hypothesis, in contrast to the hypothesis of acquired immunity, predicts a distribution of the number of resistant bacteria with a long tail of high numbers of resistant bacteria, the determination of the *variance* of the distribution should be helpful in differentiating between the two hypotheses. We may here again determine first the true variance—that is, the variance of the complete distribution—and second the likely variance in a limited number of cultures, by omitting those cases which are not likely to occur in a limited number of cultures.

The variance may be calculated in a simple manner by considering separately the variances of the partial distributions of resistant bacteria, each partial distribution comprising the resistant bacteria belonging to clones of one age group. The distribution of the total number of resistant bacteria is the resultant of the superposition of these independent partial distributions.

Each partial distribution is due to the mutations which occurred during a certain time interval $d\tau$, extending from $(t-\tau)$ to $(t-\tau+d\tau)$. The average number of mutations which occurred during this interval is, according to equation (3),

$$dm = aN_\tau d\tau = aN_t e^{-\tau} d\tau. \tag{9}$$

These mutations will be distributed according to Poisson's law, so that the variance of each of these distributions is equal to the mean of the distribution. We are however not interested in the distribution of the number of mutations but in the distribution of the number of resistant bacteria which stem from these mutations at the time of observation—that is, after the time interval τ. Each original mutant has then grown into a clone of size e^τ. The distribution of the resistant bacteria stemming from mutations occurred in the time interval $d\tau$ has therefore an average value which is e^τ times greater than the average number of mutations, and a variance which is $e^{2\tau}$ times greater than the variance of the number of mutations. Thus we find for the average number of resistant bacteria:

$$d\rho = aN_t d\tau,$$

and for the variance of this number

$$\mathrm{var}_{d\rho} = aN_t e^\tau d\tau.$$

From this variance of the partial distribution, the variance of the distribution of all resistant bacteria may be found simply by integrating over the appropriate time interval—that is, either from time t to time o (τ from o to t), if the true variance is wanted, or from time t to time t_0 (τ from o to $t-t_0$), if the likely variance in a limited number of cultures is wanted. In the first case we obtain:

$$\mathrm{var}_\rho = aN_t(e^t - 1). \tag{10}$$

In the second case we obtain:

$$\mathrm{var}_r = aN_t[e^{(t-t_0)} - 1]. \tag{10a}$$

Substituting here the previously found value of $(t-t_0)$ and neglecting the second term in the brackets, we obtain:

$$(11) \qquad var_r = Ca^2N_t^2.$$

Comparing this value of the likely variance with the value of the likely average, from equation (8), we see that the ratio of the standard deviation to the average is:

$$(12) \qquad \sqrt{var_r}/r = \sqrt{C}/\ln (N_tCa).$$

It is seen that this ratio depends on the logarithm of the mutation rate and will consequently be only a little smaller for mutation rates many thousand times greater than those considered in the experiments reported in this paper.

In the beginning of this theoretical discussion we pointed out that the hypothesis of acquired immunity leads to the prediction of a distribution of the number of resistant bacteria according to Poisson's law, and therefore to the prediction of a variance equal to the average. On the other hand, if we compare the average, equation (8), with the variance, equation (11), (not, as above, with the square root of the variance), we obtain

$$(12a) \qquad var_r = rN_tCa/\ln (N_tCa).$$

Equation (12a) shows that the likely ratio between variance and average is much greater than unity on the hypothesis of mutation, if (N_tCa), the total number of mutations which occurred in our cultures, is large compared to unity.[5]

It is possible to carry the analysis still further and to evaluate the higher moments of the distribution function of the number of resistant bacteria, or even the distribution function itself. The moments are comparatively easy to obtain, while the calculation of the distribution function involves considerable

[5] In some of the experiments reported in the present paper we did not determine the total number of resistant bacteria in each culture, but the number contained in a small sample from each culture. In these cases the variance of the distribution of the number of resistant bacteria will be slightly increased by the sampling error. The proper procedure is here first to find the average number of resistant bacteria per culture by multiplying the average per sample by the ratio

$$(13) \qquad \frac{\text{volume of culture}}{\text{volume of sample}},$$

second, to evaluate the mutation rate with the help of equation (8); third, to figure the likely variance for the cultures by equation (11); fourth, to divide this variance by the square of the ratio (13) to obtain that part of the variance in the samples which is due to the chance distribution of the mutations. The experimental variance should be greater than this value, on account of the sampling variance. The sampling variance is in all our cases only a small correction to the total variance, and it is sufficient to use its upper limit, that of the Poisson distribution, in our calculations. Consequently, when comparing the experimental with the calculated values, we first subtract from the experimental value the sampling variance, which we take to be equal to the average number of resistant bacteria.

mathematical difficulties. An approximation to the beginning of the distribution function—that is, to its values for small numbers of resistant bacteria—may be obtained by grouping mutations according to the bacterial generation during which they occurred. For instance, the probability of obtaining seven resistant bacteria may be broken down into the sum of the following alternative events: (a) seven mutations during the last generation; (b) three mutations during the last generation and two mutations one generation back; (c) three mutations during the last generation and one mutation two generations back; (d) one mutation during the last generation and three mutations one generation back; (e) one mutation during the last generation, one mutation one generation back and one mutation two generations back.

The probability of each of these events depends only on the mutation rate and on the final number of bacteria.

The grouping of mutations according to the bacterial generation during which they occurred, and the assumption that the bacteria increase in simple geometric progression, simplify the calculation sufficiently to permit numerical computation. On the other hand, the classes with two, four, eight, etc., mutants are artificially favored by this procedure, so that a somewhat uneven distribution results, with too high values for two, four, eight, etc., resistant bacteria (see fig. 2).

MATERIAL AND METHODS

The material used for our experimental study consisted of a bacterial virus α and of its host, *Escherichia coli* B (DELBRÜCK and LURIA 1942). Secondary cultures after apparently complete lysis of B by virus α show up within a few hours from the time of clearing. They consist of cells which are resistant to the action of virus α, but sensitive to a series of other viruses active on B. The resistant cells breed true and can be established easily as pure cultures. No trace of virus could be found in any pure culture of the resistant bacteria studied in this paper. The resistant strains are therefore to be considered as non-lysogenic.

Tests were made to see whether the resistance to virus α was a stable character of the resistant strains. In the first place, it was found that virus α is not appreciably adsorbed by any of the resistant strains. In the second place, when a certain amount of virus α is mixed with a growing culture of a resistant strain, no measurable increase of the titer of virus α occurs over a period of several hours. This is a very sensitive test for the occurrence of sensitive bacteria, and its negative result for all resistant strains shows that reversion to sensitivity must be a very rare event.

Morphologically at least two types of colonies of resistant bacteria may be distinguished. The first type of colony is similar to the type produced by the sensitive strain both in size and in the character of the surface and of the edge. The second type of colony is much smaller and translucent. The difference in colony type is maintained in subcultures. Microscopically the bacteria from these two types of colonies are indistinguishable. They also do not differ

from each other or from the sensitive strain in their fermentation reactions on common sugars and in the characteristics of their growth-curves in nutrient broth. In particular, the lag periods, the division times during the logarithmic phase of growth, and the maximum titers attained are identical for the sensitive strain and for the two variants. Both variants, therefore, fulfill the requirements for the applicability of the theory developed above.

In the presentation of our experimental results we have lumped the counts of the two types of colonies together, because: (1) theoretically, this is equivalent to summing the corresponding mutation rates; (2) experimentally, we are not certain whether each of these types does not actually comprise a diversity of variants; (3) experimentally, no correlation appeared to exist between the occurrence of these variants, which shows the independence of the causes of their occurrence.

Cultures of B were grown either in nutrient broth (containing .5 percent NaCl) or in an asparagin-glucose synthetic medium. In the latter, the division time during the logarithmic phase of growth was 35 minutes, as compared with 19 minutes in broth. In synthetic medium, the acidity increased during the time of incubation from pH 7 to pH 5.

In cultures of strain B, between 10^{-8} and 10^{-5} of the bacteria are found usually to give colonies resistant to the action of virus α when samples of such cultures are plated with large amounts of virus. In order to be reasonably certain that the resistant bacteria found in the test had not been introduced into the test culture with the initial inoculum, the test cultures were always started with very small inocula, containing between 50 and 500 bacteria from a growing culture. Thus any resistant bacterium found at the moment of testing (when the culture contains between 10^8 and 5×10^9 bacteria/cc) must be an offspring of one of the sensitive bacteria of the inoculum.

All platings were made on nutrient agar plates. The plating experiments for counting the number of resistant bacteria in a liquid culture of the sensitive strain were done by plating either a portion or the entire culture with a large amount of virus α. The virus was plated first, and spread over the entire surface of the agar. A few minutes later the bacterial suspension to be tested was spread over the central part of the plate, leaving a margin of at least one centimeter. Thus all bacteria were surrounded by large numbers of virus particles.

Microscopic examination of plates seeded in this manner showed that lysis takes place very quickly; only bacteria which at the time of plating were in the process of division may sometimes complete the division. The resistant colonies which appear after incubation are therefore due to resistant bacterial cells present at the time of plating.

The total number of bacteria present in the culture to be tested was determined by colony counts in the usual manner.

The resistant colonies of the large type appear after 12–16 hours of incubation, the colonies of the small type appear after 18–24 hours, and never reach half the size of the former ones. Counts were usually made after 24 and 48 hours.

EXPERIMENTAL

A Test of the Reliability of the Plating Method

In our experiments we wanted to study the fluctuations of the numbers of resistant bacteria found in cultures of sensitive bacteria. It was therefore necessary to show first that the method of testing did not involve any unrecognized variables, which caused the number of resistant colonies to vary from plate to plate or from sample to sample.

Therefore, parallel platings were made using a series of samples from the same bacterial culture. If our plating method is reliable, fluctuations should in this arrangement be due to random sampling only, and the variance from a series of such samples should be equal to the mean.

Table 1 gives the results of three such experiments. It will be seen that in

TABLE 1

The number of resistant bacteria in different samples from the same culture.

SAMPLE NO.	EXP. NO. 10a RESISTANT COLONIES	EXP. NO. 11a RESISTANT COLONIES	EXP. NO. 3 RESISTANT COLONIES
1	14	46	4
2	15	56	2
3	13	52	2
4	21	48	1
5	15	65	5
6	14	44	2
7	26	49	4
8	16	51	2
9	20	56	4
10	13	47	7
mean	16.7	51.4	3.3
variance	15	27	3.8
χ^2	9	5.3	12
P	.4	.8	.2

all three cases variance and mean agree as well as may be expected. There is therefore no reason to assume that the method of sampling or plating introduces any fluctuations into our results besides the sampling error.

Fluctuations of the Number of Resistant Bacteria in Samples from a Series of Similar Cultures

As pointed out in the introduction and in the theoretical part, the hypothesis of acquired immunity and the hypothesis of mutation lead to radically different predictions regarding the distribution of the number of resistant bacteria in a series of similar cultures. The hypothesis of acquired immunity predicts a variance equal to the average, as in sampling, while the mutation hypothesis predicts a much greater variance.

Series of five to 100 cultures were set up in parallel with small equal inocula, and were grown until maximum titer was reached. Three kinds of cultures

were used—namely: (1) 10.0 cc aerated broth cultures; (2) .2 cc broth cultures; (3) .2 cc synthetic medium cultures.

The results of all tests for the number of resistant bacteria are summarized in table 2 and table 3.

TABLE 2

The number of resistant bacteria in series of similar cultures.

EXPERIMENT NO.	1	10	11	15	16	17	21a	21b
Number of cultures	9	8	10	10	20	12	19	5
Volume of cultures, cc	10.0	10.0	10.0	10.0	.2*	.2*	.2	10.0
Volume of samples, cc	.05	.05	.05	.05	.08	.08	.05	.05
Culture No.								
1	10	29	30	6	1	1	0	38
2	18	41	10	5	0	0	0	28
3	125	17	40	10	3	0	0	35
4	10	20	45	8	0	7	0	107
5	14	31	183	24	0	0	8	13
6	27	30	12	13	5	303	1	
7	3	7	173	165	0	0	0	
8	17	17	23	15	5	0	1	
9	17		57	6	0	3	0	
10			51	10	6	48	15	
11					107	1	0	
12					0	4	0	
13					0		19	
14					0		0	
15					1		0	
16					0		17	
17					0		11	
18					64		0	
19					0		0	
20					35			
Average per sample	26.8	23.8	62	26.2	11.35	30	3.8	48.2
Variance (corrected for sampling)	1217	84	3498	2178	694	6620	40.8	1171
Average per culture	5360	4760	12400	5240	28.4	75	15.1	8440
Bacteria per culture	3.4×10^{10}	4×10^{10}	4×10^{10}	2.9×10^{10}	5.6×10^{8}	5×10^{8}	1.1×10^{8}	3.2×10^{10}
Mutation rate	1.8×10^{-8}	1.4×10^{-8}	4.1×10^{-8}	2.1×10^{-8}	1.1×10^{-8}	3.0×10^{-8}	3.3×10^{-8}	3.0×10^{-8}
Standard deviation / Average (exp.)	1.3	.39	.95	1.8	2.3	2.7	1.7	.71
Standard deviation / Average (calc.)	.35	.33	.33	.37	.94	.67	1.04	.26

* Cultures in synthetic medium.

It will be seen that in every experiment the fluctuation of the numbers of resistant bacteria is tremendously higher than could be accounted for by the sampling errors, in striking contrast to the results of plating from the same culture (see table 1) and in conflict with the expectations from the hypothesis of acquired immunity.

We want to see next whether these results fit the expectations from the hypothesis of mutation. We must therefore compare the experimental results with the relations developed in the theoretical part, keeping in mind that the theory contains several simplifying assumptions.

First we can compare, according to equation (12), the experimental and the calculated values of the ratio between the standard deviation and the average of the numbers of resistant bacteria. These ratios are included in tables 2 and 3. It is seen that the experimental and theoretical values are reasonably close.

However, in all but one case the experimental ratio is greater than the value calculated from the theory—that is, the variability is even greater than predicted.

TABLE 3

Distribution of the numbers of resistant bacteria in series of similar cultures.

EXPERIMENT NO.	22		23	
Number of cultures	100		87	
Volume of cultures, cc	.2*		.2*	
Volume of samples, cc	.05		.2	
	Resistant bacteria	*Number of cultures*	*Resistant bacteria*	*Number of cultures*
	0	57	0	29
	1	20	1	17
	2	5	2	4
	3	2	3	3
	4	3	4	3
	5	1	5	2
	6– 10	7	6– 10	5
	11– 20	2	11– 20	6
	21– 50	2	21– 50	7
	51– 100	0	51– 100	5
	101– 200	0	101– 200	2
	201– 500	0	201– 500	4
	501–1000	1	501–1000	0
Average per sample	10.12		28.6	
Variance (corrected for sampling)	6270		6431	
Average per culture	40.48		28.6	
Bacteria per culture	2.8×10^8		2.4×10^8	
Mutation rate	2.3×10^{-8}		2.37×10^{-8}	
Standard deviation / Average: exp.	7.8		2.8	
Standard deviation / Average: calc.	1.5		1.5	

* Cultures in synthetic medium.

A part of this discrepancy may be accounted for by the fact that the time t_0, mutations occurring prior to which were disregarded by the theory, was chosen in such a manner that on the average one mutation would occur prior to time t_0. This mutation, if it occurs, will of course tend to increase the variance, and in some of the experiments the high value of the experimental variance can be traced directly to one exceptional culture in which a mutation had evidently occurred several generations prior to time t_0. Unfortunately, there is no general criterion by which one might eliminate such cultures from the statistical analysis, because, in a culture with an exceptionally high count of resistant bacteria, these do not necessarily stem from one exceptionally early mutation, but may also be due to an exceptionally large number of mutations after time t_0.

There may also be other reasons why the observed variances are higher than the expected ones. First of all, the simplifying assumption that the mutation

rate per bacterial generation is independent of the physiological state of the bacteria may be too simple. If the mutation rate is higher for actively growing bacteria than for bacteria near the saturation limit of the cultures, early mutations and big clone sizes will be favored, and therefore higher variations of the numbers of resistant bacteria can be expected. Second, the assumption of a sudden transition from sensitivity to resistance may also be too simple. It is conceivable that the character "resistance to virus" may not fully develop in the bacterial cell in which the mutation occurs, but only in its offspring, after one or more generations. However, if this were the case, cultures with only one or two resistant bacteria should be relatively rare. The last experiment listed in table 3, in which the entire cultures were plated, shows a rather high proportion of cultures with only one resistant bacterium. This seems to show that the

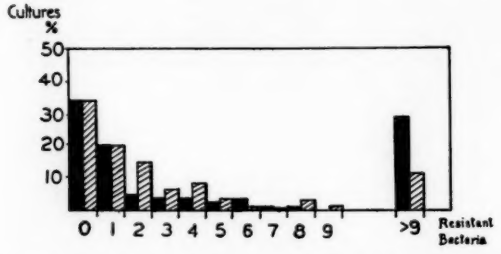

FIGURE 2.—Experimental (Experiment No. 23) and calculated distributions of the numbers of resistant bacteria in a series of similar cultures. Solid columns: experimental. Cross-hatched columns: calculated.

character "resistance to virus" in general does come to expression in the bacterial cell in which the corresponding mutation occurred, as assumed by the theory.

Another way of comparing the experimental results with the theory is to compare the experimental distribution of resistant bacteria with the approximate distribution calculated by the method outlined at the end of the theoretical part. The theoretical distribution has to be calculated from the average number of mutations per culture given by equation (5). Only experiments where the whole culture is tested can therefore be used for such a comparison. This method tests the fitting of the expectations for small numbers of resistant bacteria, in contrast to the comparison of the standard deviations, which involves predominantly the cultures with high numbers of resistant bacteria.

Figure 2 shows the experimental and calculated distributions for Experiment No. 23; the cultures with more than nine resistant bacteria are lumped together in one class, since the distribution has not been calculated for values higher than nine.

It is seen that the fitting for small values is satisfactory. In particular, the

number of cultures with one resistant bacterium very closely fits the expectation. The classes with two, four, eight, etc., resistant bacteria are bound to be favored in the theoretical distribution, as explained in the theoretical part.

The results shown in figure 2 also confirm the assumption that the discrepancy between experimental and calculated standard deviations must be due to an excess of cultures with large numbers of resistant bacteria.

Summing up the evidence, we may say that the experiments show clearly that the resistant bacteria appear in similar cultures not as random samples but in groups of varying sizes, indicating a correlating cause for such grouping, and that the assumption of genetic relatedness of the bacteria of such groups offers the simplest explanation for them.

Mutation Rate

As pointed out in the theoretical part of this paper, mutation rates may be estimated from the experiments by two essentially different methods. The first method makes use of the fact that the number of mutations in a series of similar cultures should be distributed in accordance with Poisson's law; the average number of mutations per culture is calculated from the proportion of cultures containing no resistant bacteria at the moment of the test, according to equation (5).

There are two technical difficulties involved in the application of this method. In the first place, rather large numbers of cultures have to be handled and conditions have to be chosen so that the proportion of resistant bacteria is neither too small nor too large. In the second place, the entire cultures have to be tested, which means, in our method of testing, that cultures of rather small volume have to be used and great care must be taken to plate as nearly as possible the entire culture.

Experiment No. 23 (see table 3) permits an estimate of the mutation rate by this method. Out of 87 cultures, no resistant bacteria were found in 29 cultures, a proportion of .33. From equation (5) we calculate therefore that the average number of mutations per culture in this experiment was 1.10. Since the total number of bacteria per culture was 2.4×10^8, we obtain as the mutation rate, from equation (4),

$$a = .47 \times 10^{-8} \text{ mutations per bacterium per time unit}$$

$$= .32 \times 10^{-8} \text{ mutations per bacterium per division cycle.}$$

This calculation makes use exclusively of the proportion of cultures containing no resistant bacteria. It is therefore inefficient in its use of the information gathered in the experiment.

The second method makes use of the average number of resistant bacteria per culture. The relation of this average number with the mutation rate was discussed in the theoretical part of this paper and was found to be expressed by equation (8). The mutation rates calculated by this method for each experiment are collected in table 4.

TABLE 4

Values of mutation rate from different experiments.

EXPERIMENT NO.	NUMBER OF CULTURES	VOLUME OF CULTURES	MUTATION RATE
		cc	*Mutations per bacterium per time unit*
1	9	10.0	1.8×10^{-8}
10	8	10.0	1.4×10^{-8}
11	10	10.0	4.1×10^{-8}
15	10	10.0	2.1×10^{-8}
16	20	.2*	1.1×10^{-8}
17	12	.2*	3.0×10^{-8}
21a	19	.2	3.3×10^{-8}
21b	5	10.0	3.0×10^{-8}
22	100	.2*	2.3×10^{-8}
23	87	.2*	2.4×10^{-8}
Average			2.45×10^{-8}

* Cultures in synthetic medium.

It will be seen that the values of the mutation rate obtained by the second method are all higher than the value found by the first method. This discrepancy may be traced back to the same cause as the discrepancy between the calculated and observed values of the standard deviation of the numbers of resistant bacteria. This, we found, was due to an excess of early mutations, giving rise to big clones of resistant bacteria. These big clones do not affect the mutation rate calculated by the first method, but they do affect the results of the second method, which is based on the average number of resistant bacteria.

One sees in table 4 that the mutation rate calculated by the second method does not vary greatly from experiment to experiment. In particular, it will be noted that there is no significant difference between the values obtained from cultures in broth and from cultures in synthetic medium, notwithstanding the considerable difference of metabolic activity and of growth rate of the bacteria in these two media. This shows that the simple assumption of a fixed small chance of mutation per physiological time unit is vindicated by the results. It may also be noted in table 4 that there is no significant difference between the mutation rates obtained from 10 cc cultures and those obtained from .2 cc cultures, or between the experiments with many and those with few cultures. The variability of the value of the mutation rate seems to be solely due to the peculiar probability distribution of the number of resistant bacteria in series of similar cultures predicted by the mutation theory.

At this point an experiment may be mentioned by which it was desired to find out whether or not mutations occur in a culture after the bacteria have ceased growing. A culture was grown to saturation and was then tested repeatedly for resistant bacteria and for total number of bacteria over several

days. The proportion of resistant bacteria did not change, even when the sensitive bacteria began to die, showing that the resistant bacteria have the same death rate in aging cultures as the sensitive bacteria.

DISCUSSION

We consider the above results as proof that in our case the resistance to virus is due to a heritable change of the bacterial cell which occurs independently of the action of the virus. It remains to be seen whether or not this is the general rule. There is reason to suspect that the mechanism is more complex in cases where the resistant culture develops only several days after lysis of the sensitive bacteria.

The proportion of mutant organisms in a culture and the mutation rate are far smaller in our case than in other studied cases of heritable bacterial variation. The possibility of investigation of such rare mutations is in our case merely the result of the method of detecting the mutant organisms. In other cases, the variants are detected by changes in the colony type which is produced by the mutant organism, either in the pigmentation or in the character of the surface or the edge of the colony. Often, colonies of intermediate character occur, and it is difficult to decide whether they are mixed colonies or stem from bacteria with intermediate character. This is particularly true of cases where the mutation rate is high and where reverse mutation occurs. Fairly high mutation rates, however, are a prerequisite of any study of colony variants, since the number of colonies that can be examined is limited by practical reasons.

The study of mutations causing virus resistance is free of these difficulties. The segregation of the mutant from the normal organisms occurs in the one-cell stage by elimination of the normal individuals, and the character of the colony which develops from a mutant organism is of secondary importance. Owing to the total elimination of the normal individuals, the number of organisms which may be examined is very much higher than for any other method; more than 10^8 bacteria may be tested on a single plate. Since the mutations to virus resistance are often associated with other significant characters, the method may well assume importance with regard to the general problems of bacterial variation.

It must not be supposed that the peculiar statistical difficulties encountered in our case are restricted to cases of very low mutation rates. The essential condition for the occurrence of the peculiar distribution studied in the theoretical part of this paper is the following: ***the initial number of bacteria in a culture must be so small that the number of mutations which occur during the first division cycle of the bacteria is a small number.*** This will always be true, however great the mutation rate, if one studies cultures containing initially a small number of organisms.

In a series of very interesting studies of the color variants of *Serratia marcescens*, BUNTING (1940a, 1940b, 1942; BUNTING and INGRAHAM 1942) succeeded to some extent in obviating the statistical difficulties by always using

inocula of about 100,000 bacteria. In some of her cases this number was sufficiently high to result in numerous mutations during the first division cycle of the bacteria. In other cases the number was apparently not high enough, since the author reports troublesome variations of the fractions of variants in successive subcultures. In those cases where the size of the inocula was high enough, the author succeeded in deriving reproducible values for the mutation rates from the study of single cultures, followed through numerous subcultures. In these cases it is sufficient to apply the equations of the theory referring to the *average* numbers of mutants as a function of time. It is clear, however, that this method is applicable only in cases of mutation rates of at least 10^{-4} per bacterium per division cycle.

In our case, as in many others, the virus resistant variants do not exhibit any striking correlated physiological changes. There is therefore little opportunity for an inquiry into the nature of the physiological changes responsible for the resistance to virus. Since the offspring of the mutant bacteria, when isolated after the test, are unable to synthesize the surface elements to which the virus is specifically adsorbed in the sensitive strain, one might suppose that this loss is a direct effect of the mutation. However, it is also conceivable that the loss occurs upon contact with virus, since it is detected only after such contact (hypothesis b_1). In some of the cases studied by BURNET (1929), where the mutational change to resistance is correlated with a change of phase, from smooth to rough or vice versa, the change of the surface structure must be a direct result of the mutation, since the mutant colonies may be picked up prior to the resistance test and, when tested, exhibit the typical change of affinity of the surface structure. These findings make it more probable that the loss of surface affinity to virus is a direct effect of the mutation.

The alteration of specific surface structures due to genetic change is a phenomenon of the widest occurrence. The genetic factors determining the antigenic properties of erythrocytes are well known. There is evidence (WEBSTER 1937; HOLMES 1938; STEVENSON, SCHULTZ, and CLARK 1939) that resistance or sensitivity to virus in plants and animals is correlated with, or even dependent on, genetic changes, possibly affecting the antigenic make-up of the cellular surface. The proof that resistance to a bacterial virus may be traced to a specific genetic change may assume importance, therefore, with regard to the general problems of virus sensitivity and virus resistance.

SUMMARY

The distribution of the numbers of virus resistant bacteria in series of similar cultures of a virus-sensitive strain has been analyzed theoretically on the basis of two current hypotheses concerning the origin of the resistant bacteria.

The distribution has been studied experimentally and has been found to conform with the conclusions drawn from the hypothesis that the resistant bacteria arise by mutations of sensitive cells independently of the action of virus.

The mutation rate has been determined experimentally.

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THE ORIGIN OF ISO-CHROMOSOMES AT MEIOSIS

NORMAN H. GILES, JR.
Osborn Botanical Laboratory, Yale University

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AN ISO-CHROMOSOME is a chromosome having two identical arms (DARLINGTON 1939). Such chromosomes have been studied recently by RHOADES (1940) in Zea and by DARLINGTON (1940) in Fritillaria. It is also very probable that the extra chromosomes in secondary trisomics, such as the numerous types which have been found in Datura (BLAKESLEE and AVERY 1938) are true iso-chromosomes. The manner in which these metacentric iso-chromosomes arise is of considerable cytogenetical interest. In Zea it has been clearly demonstrated that telocentric chromosomes give rise to iso-chromosomes. Furthermore it is known (UPCOTT 1937; KOLLER 1938; DARLINGTON 1939) that telocentric chromosomes result from the aberrant division—misdivision—of the centromere in univalent chromosomes at meiosis. Consequently it has been inferred that iso-chromosomes are derived from telocentric chromosomes which themselves result from misdivision. Recently in Fritillaria DARLINGTON (1940) has observed this complete sequence of events cytologically. It is also possible that the telocentric chromosome types resulting from misdivision at AI of meiosis may become transformed directly into functional iso-chromosomes through a rotation of their two identical arms such that the centromere assumes a median position. However, the origin of iso-chromosomes in this manner and their presence in microspore nuclei after surviving the second meiotic division have not been demonstrated cytologically.

The observations to be reported in this paper have revealed a unique type of centromere behavior in normally paired and oriented bivalents which gives rise directly to iso-chromosomes at meiosis and results in their regular presence in a considerable fraction of the resulting microspores. This anomalous centromere behavior has been found in a single diploid plant of Gasteria sp. (probably *maculata*) now in the collections of the YALE DEPARTMENT OF BOTANY. It is not known where or how this particular plant originated. Chromosome behavior has been studied at meiosis in pollen mother cells, at the first post-meiotic mitosis in microspores, and at somatic mitoses in anthers and root tips by means of acetocarmine and Feulgen smear preparations. The unusual behavior at meiosis has been observed at the time of annual flowering for two successive years and is evidently a constant character of the plant. It has not yet been possible to obtain evidence as to the manner in which the anomalous meiotic behavior is inherited, since the plant is self-sterile, and no other individuals have been available for making cross-pollinations.

OBSERVATIONS AT MITOSIS IN THE MICROSPORES

The first indications of possible aberrant chromosome behavior in this specimen of Gasteria were obtained from observations at the first post-meiotic mitosis in the microspore. It was found that two distinct kinds of microspores

with respect to chromosome complements are present. One (fig. 1; plate I, fig. B) contains seven chromosomes which may be classified into three general categories: (1) three long chromosomes with subterminal centromeres (referred to hereafter as STL chromosomes); (2) three short chromosomes with subterminal centromeres (referred to as STS chromosomes); and (3) one long chromosome with a submedian centromere and a trabant (satellite) on the long arm (referred to as the SML chromosome). This is the normal haploid complement for the genus as originally described by TAYLOR (1924) and subsequently confirmed by other cytologists. The second kind of microspore (fig. 2; plate I, fig. A) also has seven chromosomes, but one is a new type. The usual three STL and three STS chromosomes are present, but the SML chromosome is absent. In its place is a long chromosome with an exactly median centromere and with trabants on each of its arms (referred to as the ML chromosome). The ML chromosome appeared to be an iso-chromosome, its two arms being identical with the long arm of the SML chromosome present in the other microspores. This chromosome type divides and separates normally at the microspore mitosis (plate I, fig. C). The relative frequencies of the two types of chromosome complements in the microspores were determined by chromosome counts at metaphase or anaphase. These frequencies are not constant for all stages in the pollen cycle but vary in a regular way, as the results obtained from a count of 389 cells (table 1) indicate. The percentages

TABLE 1

Relative frequencies of the two types of chromosome complements in pollen grains during different stages of the pollen cycle. All nuclei with more than seven chromosomes as a result of occasional nondisjunction at AI have not been included in counts. See text for explanation of chromosome symbols.

STAGE OF MAJORITY OF POLLEN NUCLEI	NO. CELLS WITH SPECIFIC CHROMOSOME TYPE		% ML
	SML (NORMAL)	ML (ISO-CHROMOSOME)	
Prophase	115	9	7.3
Metaphase and anaphase	94	50	34.7
Telophase or later	55	66	54.5
Totals	264	125	32.2

show that nuclei containing an ML chromosome undergo mitosis later in the pollen cycle than do those with an STL chromosome. Microspores containing an ML chromosome are evidently retarded in their development. Because of this differential rate of development of the two types of microspores, it is difficult to make an accurate estimate of the percentage of spores carrying the ML chromosome. However, in obtaining the data for table 1, approximately equal numbers of microspores were studied at the three stages indicated, and the results suggest that the ratio of microspores with SML chromosomes to those with ML chromosomes is about 2 to 1.

Observations of fully mature pollen grains indicate that these are divisible into three relatively distinct classes: (1) large grains staining darkly with acetocarmine and having two well differentiated nuclei; (2) grains intermediate in size with less deeply staining cytoplasm and not so clearly differentiated nuclei; and (3) small, empty grains. An average of counts of several hundred pollen grains from several anthers gave the following percentages for these three types: (1) 42 percent, (2) 24 percent, (3) 34 percent.

OBSERVATIONS AT MEIOSIS

On the basis of the two types of complements in the microspores it was at first thought that the plant was heterozygous, having one SML and one ML chromosome. Examination of its diploid complement at mitosis in anther and root tip cells showed, however, that chromosomes of the normal number and morphology are present in the somatic cells (fig. 3). Furthermore, in none of the mitoses examined was there evidence of abnormal chromosome division or separation. It then seemed probable that the new chromosome type resulted from some sort of unusual meiotic chromosome behavior. Observations at meiosis did indeed reveal an exceedingly anomalous behavior of the centromeres of the SML chromosomes in a considerable fraction of the pollen mother cells. This unusual behavior does not become apparent until first anaphase (AI). Prophase and metaphase stages appear to be entirely normal. Pairing and chiasmata formation take place, followed by regular orientation (fig. 4) of all the tetrads (bivalents) on the metaphase plate. When early anaphase separation begins, only the SML tetrad behaves in an aberrant manner. The other six tetrads continue through both meiotic divisions in an entirely normal fashion, aside from occasional instances of non-disjunction at AI.

The SML tetrad exhibits at least three distinct types of behavior at AI. In type I (plate I, fig. D, E) the disjunction of the tetrad appears, at least superficially, to be normal. The two dyads pass to opposite poles, each composed of two long arms and two short arms. In some cells (type Ia; plate I, fig. E) it is clear that the four arms are parts of two normal chromatids—each having one long and one short arm—held together as usual by the single centromere. In some of the other cells it appears, however, that the four arms of each dyad are not united to form two normal chromatids. Rather some of the configurations suggest that the two long arms and the two short arms of a dyad may have somehow become united to form two iso-chromatids held together by the single centromere. Either one (type Ic; fig. 5) or both (type Ib; fig. 6) dyads in a single cell may give this impression. However, it is not as yet possible to be absolutely certain of these various types and to classify all cells accordingly. Therefore all cells with both dyads having two long and two short arms have been recorded as type I. In type II (plate I, fig. F) an exceedingly bizarre kind of division and disjunction of the centromeres of the SM tetrad has occurred, giving rise to two unlike, asymmetrical dyads. One of these dyads has one short and three long arms attached to its centromere; to the centromere of the opposite dyad are attached one long and three short arms—the remaining arms of the tetrad. In type III (plate I, fig. G) still

another aberrant kind of division and disjunction of the centromeres has taken place, giving rise to two unlike but symmetrical dyads. One has four long arms attached to its centromere, the other, four short arms attached to its centromere. The relative frequencies of these three types are shown in table 2, which

TABLE 2

Frequencies of the three distinct types of anaphase I behavior of the SML tetrad. For descriptions and illustrations of the three types see text and plate I, figures D–G.

TYPE OF BEHAVIOR AT AI	NO. OF CELLS	PERCENT (%)
I	210	48.8
II	180	41.8
III	40	9.4
Totals	430	100.0

includes an analysis of 430 cells from several different flowers. Only late anaphase stages have been scored in order to avoid any possible misclassifications of types. Types I and II are both common and do not differ greatly in frequency, while type III occurs in less than 10 percent of the cells.

Chromosome behavior during the second meiotic division was next studied. As far as can be judged from observations at second metaphase of meiosis (MII) and at early second anaphase (AII) the centromeres of dyads derived from the SML tetrad at AI divide normally in the expected mitotic fashion, with the plane of division parallel to the long axis of the chromosome (fig. 7). The two chromosomes derived from each dyad then pass in a regular manner to opposite poles. As a consequence of the aberrant behavior at meiosis many of the quartets produced at AII contain one or more iso-chromosomes. These iso-chromosomes are of two types—one has both long arms of the SML chromosome and is clearly equivalent to the ML chromosome observed previously in the pollen, the other complementary type has both short arms of the SML chromosome (this type is referred to as the MS chromosome). Furthermore it was found that there are five different kinds of quartets with respect to the types of chromosomes present and their arrangement at AII. These have been designated types A–E, and are illustrated in figures 8 to 12 and in figure 15. The quartet types at AII may be briefly described as follows: (1) type A (fig. 8) has four normal SML chromosomes arranged in the regular manner at the poles; (2) type B (fig. 9) contains two normal and both kinds of iso-chromosomes, the two iso-chromosomes being opposite normal chromosomes (that is, in the same half of the quartet); (3) type C (fig. 10) contains only iso-chromosomes, with similar types at opposite poles; (4) type D (fig. 11) resembles C except that the two unlike iso-chromosomes are at opposite poles; (5) type E (fig. 12) is similar to B in having three different chromosome types, but in this case the two normal chromosomes and the two unlike iso-chromosomes are opposite each other. The relative frequencies of these five types as obtained from an analysis of 184 cells at AII from several different flowers are

given in table 3. Type B is the most common, more than half the quartets being in this category; and type D is the least common, since less than six percent of the quartets are included in this class.

TABLE 3

Frequencies of the five types of quartets at AII. For descriptions and figures of these types see text and figures 8–12.

TYPE OF QUARTET	NO. OF CELLS	PERCENT (%)
A	35	19.1
B	98	53.2
C	13	7.1
D	10	5.4
E	28	15.2
Totals	184	100.0

DISCUSSION

In this specimen of Gasteria it is clear that two complementary types of iso-chromosomes (in addition to the normal chromosome with a subterminal centromere) are regularly formed in considerable numbers at meiosis as a result of the aberrant behavior of one of the chromosome pairs (the SML tetrad). These chromosomes are transmitted in a regular fashion to the resulting microspore nuclei such that each microspore receives only one of the three types. Since the other chromosomes behave normally throughout meiosis, all microspores have the usual haploid number of seven (aside from occasional

EXPLANATION OF PLATE I

All photomicrographs ×810

FIGURE A–C. Chromosomes at the first post-meiotic mitosis in the microspore.

FIGURE A. Microspore with six normal chromosomes and one ML iso-chromosome (indicated by arrow) at metaphase.

FIGURE B. Normal haploid complement at metaphase with one SML chromosome (indicated by arrow), three STS, and three STL chromosomes. (Cf. fig. 1 and text.)

FIGURE C. Microspore showing two ML iso-chromosomes (indicated by arrows) at anaphase.

FIGURE D–G. Chromosomes at anaphase I of meiosis.

FIGURE D. Type I configuration. Regular separation of all seven dyads. SML dyads indicated by arrows.

FIGURE E. Type I configuration. Both SML dyads (indicated by arrows) have passed to upper pole. Each is composed of two normal chromatids attached at the single centromere (type Ia).

FIGURE F. Type II configuration. Asymmetrical dyads derived from SML tetrad are present at opposite poles. Dyad at upper pole (arrow) has one long and three short arms; that at lower pole (arrow) has one short and three long arms.

FIGURE G. Type III configuration. Symmetrical dyads derived from SML tetrad are present at opposite poles. Dyad at upper pole (arrow) has four short arms; that at lower pole (arrow) has four long arms.

PLATE I

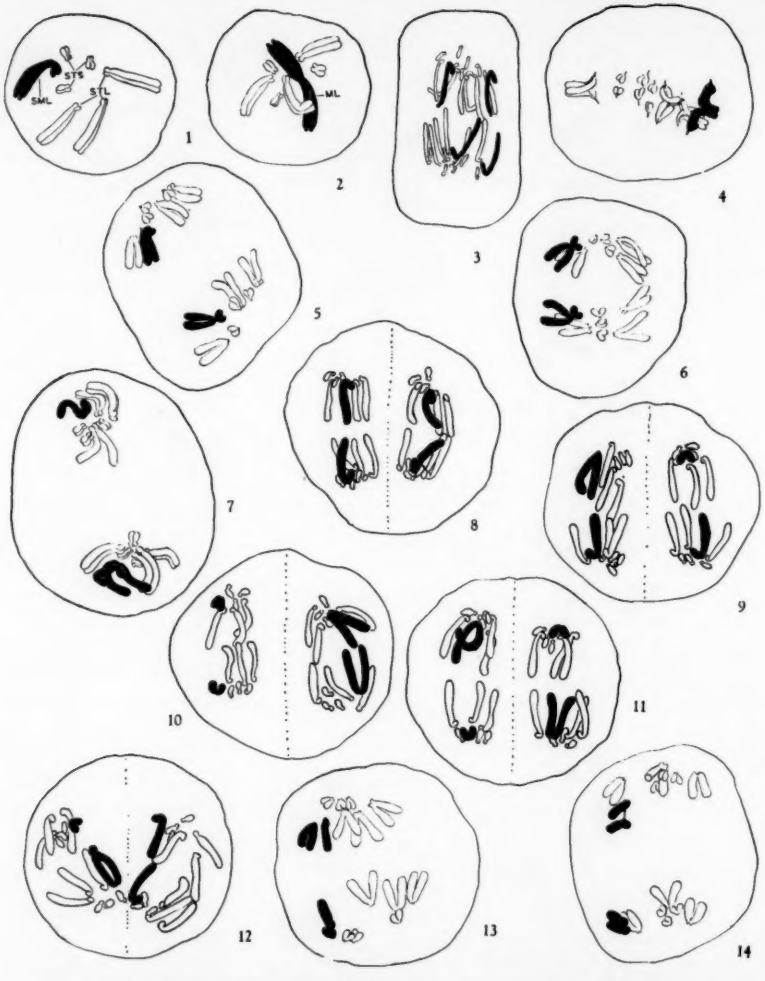

FIGURE 1. Normal haploid complement of Gasteria at metaphase of first post-meiotic mitosis. The three categories of chromosomes as to morphology are indicated by the abbreviations used in the text. ×700.

FIGURE 2. Microspore mitosis showing iso-chromosome (ML type) at metaphase. ×700.

FIGURE 3. Mitotic anaphase in root-tip cell showing regular number ($2n = 14$), morphology, and behavior of chromosomes. SML chromosomes in black. ×437.

FIGURE 4. Early anaphase I of meiosis. Evidence of regular chiasma formation, orientation, and disjunction of all the tetrads is shown. The SML tetrad is indicated. ×525.

FIGURE 5. Late anaphase I of meiosis. Type Ic configuration. Dyad from SML tetrad at upper pole (black) shows normal chromatid relations; that at lower pole shows apparent union of like arms to produce two iso-chromatids. ×525.

cells which receive a greater or lesser number due to infrequent lagging or non-disjunction of some of the chromosomes at AI). Thus there result three distinct kinds of microspores, each of which contains, in addition to three STL and three STS chromosomes, either (1) a normal SML chromosome, (2) an ML iso-chromosome, or (3) an MS iso-chromosome. These differences in the chromosome complements of the microspores have important genetical consequences. Clearly, spores with normal SML chromosomes have the full haploid set and should develop normally. Since iso-chromosomes are duplication-deficiency types, the two other kinds of spores, therefore, do not have fully normal haploid complements. The spores with an ML chromosome are deficient for genes in the short arm of the SML chromosome and have those in the long arm in duplicate, while spores with an MS chromosome are deficient for genes in the long arm and have those in the short arm in duplicate. Such spores should develop in an abnormal fashion if at all.

It is thus possible to explain the initial observations made at mitosis in the microspore. Clearly the metacentric (ML) chromosome present in about one third of the microspore mitoses is an iso-chromosome derived from the SML chromosome. Cells containing this iso-chromosome can develop through the microspore division, but their development is slow compared with that of spores with a normal SML chromosome. Since the MS iso-chromosome has never been detected at the microspore mitosis, even in nuclei dividing much later in the pollen cycle, it appears that a deficiency for the entire long arm of the SML chromosome is cell lethal. The observations on mature pollen grains provide further evidence as to the fate of spores with an MS chromosome. It will be recalled that three types of pollen can be distinguished; these correspond both qualitatively and quantitatively with the three different genetic types of microspores produced at meiosis. Calculations based on the

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FIGURE 6. Late anaphase I of meiosis. Type Ib configuration. Both dyads (black) from SML tetrad show apparent union of like arms to produce two iso-chromatids attached to each centromere. ×525.

FIGURE 7. Metaphase II of meiosis. Normal orientation of centromeres and chromatid arms of dyads derived from SML tetrad indicated (black). Configuration resulted from Type II at AI and will give rise to type B quartet at AII.

FIGURE 8. Anaphase II of meiosis. Type A quartet. Four normal SML chromosomes. ×525.

FIGURE 9. Anaphase II of meiosis. Type B quartet. Two different iso-chromosome types (ML, left; and MS, right) opposite normal SML chromosomes. ×525.

FIGURE 10. Anaphase II of meiosis. Type C quartet. Like iso-chromosome types at opposite poles. ×525.

FIGURE 11. Anaphase II of meiosis. Type D quartet. Unlike iso-chromosome types at opposite poles. ×525.

FIGURE 12. Anaphase II of meiosis. Type E quartet. Unlike iso-chromosomes at opposite poles in one half and normal SML chromosomes at opposite poles in the other half. ×525.

FIGURE 13. Anaphase I of meiosis. Type II configuration in which the half-centromeres of the dyad at the upper pole have failed to fuse, leaving one symmetrical and one asymmetrical telocentric chromosomes. ×525.

FIGURE 14. Anaphase I of meiosis. Type Ia configuration in which an apparently imperfect fusion of half-centromeres has occurred in dyad at upper pole. ×525.

figures in table 3 (from AII) indicate that the SML chromosome is present in about half (53.4 percent), whereas the ML and MS chromosomes each occur in about one-quarter (23.3 percent) of the young microspores. The previously recorded percentages of the three categories of mature pollen—(1) normal, 42 percent; (2) intermediate, 24 percent; and (3) empty, 34 percent—are in substantial agreement with the meiotic data, since it is evident that the third category consisting of empty, aborted pollen grains includes not only microspores receiving the MS chromosome, but also grains which fail to mature for other reasons.

The results of the aberrant behavior of the centromeres of the SML tetrad at meiosis in radically modifying both the types of microspores produced and their subsequent development are reasonably clear. It is now necessary to consider in greater detail the nature of this aberrant behavior. Though similar in certain ways to misdivision of the kind described in Tulipa (UPCOTT 1937), Pisum (KOLLER 1938) and Fritillaria (DARLINGTON 1939), the behavior in Gasteria differs in a number of fundamental respects. In the former genera the conditions for misdivision are the presence on the spindle of either univalent chromosomes at the first anaphase of meiosis or of unsplit chromosomes at the second anaphase. Any univalent, regardless of its origin, may misdivide. Such univalents are delayed in division and come onto the metaphase plate after the normal bivalents. Division of the centromere at MI may be transverse, separating pairs of sister arms, or oblique, separating three arms from one. It is possible also that free centromeres may sometimes be released. Chromosomes with unstable terminal centromeres are a common result of misdivision. In Gasteria the exceptional centromere behavior at meiosis differs in the following notable respects from misdivision in the genera previously cited: (1) tetrads (bivalents) rather than dyads (univalents) are involved at MI and AI; (2) only one (and always the same one) of the seven tetrads ever divides in an atypical manner; (3) this tetrad becomes normally oriented on the metaphase plate, and the separation of its halves at AI is synchronized with that of the normal tetrads; (4) anaphase separation at AI is always quantitatively symmetrical (one centromere with four arms going to each pole), but may be qualitatively asymmetrical (different combinations of arms may go to opposite poles); (5) telocentric chromosomes do not normally result; (6) iso-chromosomes are produced directly and are present in a considerable fraction of the resulting microspores.

How, then, do the centromeres of the SML tetrad divide at meiosis to give the types of configurations observed? It is difficult to follow the behavior of the two centromeres during prophase and metaphase of meiosis. However, it appears that they behave normally, remaining separate and undivided up to metaphase. If this is true, the ways in which they divide at MI may be deduced from the configurations present at AI. Types II and III are of most significance in this respect. Clearly type III must arise from a transverse division (that is, in a plane perpendicular to the long axis of the chromosome) of both centromeres, followed by the union of like halves from opposite centro-

meres and the passage of the resulting symmetrical dyads to opposite poles. That such a union of the halves of misdivided centromeres very probably does occur is shown by the presence of occasional cells in which union has failed, resulting in two telocentric half-dyads at a pole (fig. 13, 14). Type II arises from the transverse division of one centromere and the longitudinal division (that is, in a plane parallel to the long axis of the chromosome) of the other, followed by the union (presumably at random) of halves from opposite centromeres and the passage of the resulting asymmetrical dyads to opposite poles. On the basis of the preceding analysis, the following conclusions may be drawn concerning the structure and behavior of the centromere: (1) under certain circumstances the centromeres of normally paired and oriented tetrads may divide precociously at MI; (2) the division of each centromere may occur in either a longitudinal or a transverse plane; (3) the half-centromeres, each with two arms attached, produced by these divisions unite two by two (union taking place between either sister or non-sister halves), just as do the broken ends of chromosomes; (4) such newly formed centromeres function normally on the spindle at AI. The evidence that the halves of centromeres which have divided or misdivided precociously at MI unite with one another as do broken ends of chromosomes is of considerable interest. It may be asked why the centromere halves of misdivided univalents do not unite. Apparently they do not have the opportunity, being forced to move apart to opposite poles. When a bivalent (tetrad) divides precociously, however, separation still takes place between whole centromeres, thus facilitating the union of divided halves. It seems that the centromeres of telocentric chromosomes which have passed through a division cycle lose the power of reunion, since there is evidence that two terminal centromeres do not fuse with one another in maize (RHOADES 1940).

The fact that the centromere division is precocious, occurring at MI, and that the resulting half-centromeres can unite in various ways—either by sister or non-sister unions—makes possible the aberrant configurations found at AI. However, such precocious division and subsequent union need not necessarily lead to cytologically evident anomalous behavior. If the plane of division at MI were always longitudinal (as is normally the case when centromeres divide) in both centromeres and union of half-centromeres occurred at random, the resulting cytological configurations at AI and AII would all be normal. (The immediate genetical results would of course be different, depending on whether sister or non-sister halves united; that is, whether pre- or post-reduction at the centromere occurred.) Therefore, it is clear that the plane of centromere division is responsible, in the final analysis, for the presence of cytologically aberrant configurations. It thus becomes necessary to consider briefly how the plane of division in a centromere is determined. Unfortunately, there is as yet no adequate cytological evidence on this point. Presumably the way in which a centromere divides depends on its internal structure, but the nature of this internal structure is by no means clear It seems reasonable to suppose, however, as have DARLINGTON (1939) and

others that the two arms of an undivided chromosome are joined by some sort of fibrous connection through the centromere. If this is the case, the following series of events in the division of a centromere may be postulated. When the chromonema divides, the internal centromeric fiber also divides. The nature of the subsequent external division of the centromere (whether this occurs normally at metaphase of mitosis or at MII of meiosis, or precociously at MI of meiosis, as in the present case) is determined by the way in which these internal fibers become oriented. Normally the division of the internal fiber in each centromere results in two parallel strands across the centromere, each strand connecting the two unlike arms of the two daughter chromatids. Then the division of the centromere takes place in a plane parallel to and between these two fibers. Exceptionally, however, the fibers may become oriented in the opposite direction within the centromere so that they connect like arms of the daughter chromatids. How such an orientation comes about is not clear, but it could result from sister strand crossing over between the centromeric fibers with union after breakage taking place in an aberrant fashion between adjacent rather than between opposite ends. This transverse orientation of the internal fibers results in the subsequent transverse division of the centromere. A somewhat similar explanation of transverse centromere division involving micellar orientation within the centromere has been advanced by NEBEL (1939).

It is possible to explain all the various configurations found at AI and AII on the basis of centromere behavior of the kind just outlined. Following their division, orientation of the internal centromeric fibers occurs independently in the two centromeres of the SML tetrad. Consequently, when the two centromeres divide precociously at MI they may both divide longitudinally, both transversely, or one longitudinally and one transversely. The union of half-centromeres then follows, and the resulting dyads pass to opposite poles. (It is possible that division and reunion at MI occur at random, although the agreement between the observed and expected ratios of types on this basis is not sufficiently good to be certain that such is the case.) The original centromeric fiber connections between chromatid arms are still present in the dyads, and their orientation again determines the plane of centromere division at MII. As a result of this division either normal chromosomes or iso-chromosomes are produced, the arms of the iso-chromatids apparently having become normally aligned during the interphase as observations at MII indicate (fig. 7).

The diagram presented as figure 15 interprets the various observed types of behavior of the SML tetrad at meiosis on the basis of the preceding hypothesis, the orientation of the hypothetical internal centromeric fibers being indicated at MI and AI. The external centromere behavior in types II and III at MI and AI has already been considered, since these provide definite evidence for precocious centromere division at MI. The presumed orientations of the centromeric fibers which determine the plane of centromere division in these two types at both MI and MII are indicated. The qualitatively symmetrical disjunctions of type I at AI do not provide as clear evidence for aberrant behavior at MI. Superficially, these configurations appear normal. There is good

evidence, however, that the centromeres of a considerable fraction undergo precocious division and that the resulting half-centromeres unite with one another at MI. The evidence for this comes from the appearance of some of the dyads at AI (fig. 5, 6), the nature of which has already been discussed, and from the presence of quartets at AII of the types expected to result from such dyads at AI. Thus type Ib (fig. 6, 15) is complementary to type III, since

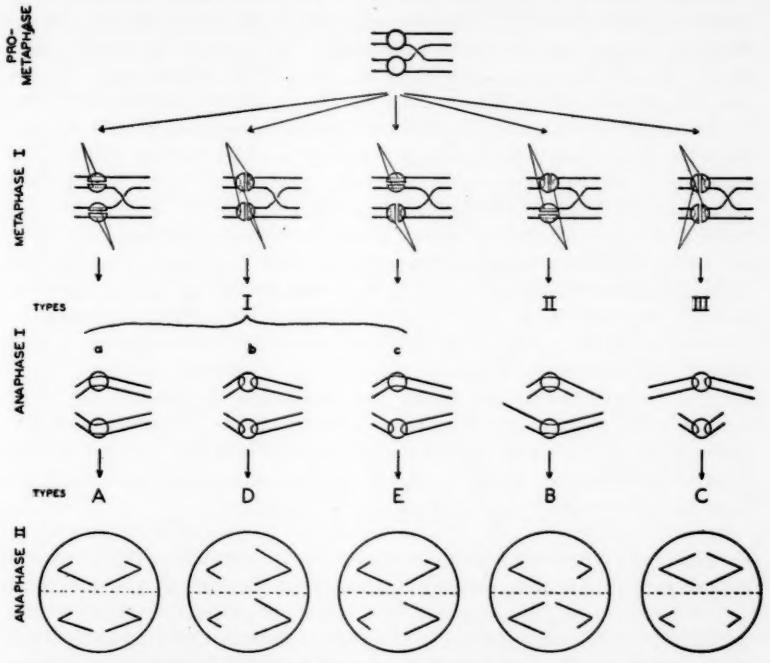

FIGURE 15. Diagram showing the various ways in which the aberrant SML tetrad behaves at meiosis. At MI the planes in which the centromeres divide precociously are indicated as are the internal centromeric fibers (by dotted lines). The pairs of joined lines above and below each type of tetrad indicate which centromere halves go together to the two poles. Types of configurations present at AI and the resulting quartet types at AII are also illustrated. For a full description of these types, and particularly for a discussion of centromere behavior at MI, see text.

both centromeres divide transversely at MI, but union occurs between ***unlike*** arms of non-sister half-centromeres (or between sister halves in the original position). Division at MII occurs parallel to and between the centromeric fibers uniting like chromatid arms, giving quartets of type D (fig. 11, 15). Type Ic (fig. 5, 15) is complementary to type II, since one centromere divides transversely, the other longitudinally, but union occurs between ***sister*** half-centromeres in the original position. Centromere division at MII then produces quartets of type E (fig. 12, 15). Type Ia and quartets of type A may of course

result from entirely normal centromere behavior through both divisions at meiosis. However, if it is assumed that precocious division always occurs at MI in the SML tetrad centromeres, these configurations may be accounted for by the longitudinal division of both centromeres and reunion of sister halves at MI followed by normal centromere division at MII.

The hypothesis which has been presented assumes that the plane of centromere division at MI is predetermined by the way in which the internal centromeric fibers become oriented during prophase of meiosis. Consequently, division at MII presumably always occurs so as to separate the same centromere halves which became united at MI. This point, however, has not been definitely established. The types of quartets found at AII do not provide critical evidence as to the way in which the centromere divides at MII. Clearly types II and III at AI can give rise respectively to types B and C only at AII, regardless of whether the plane of centromere division at MII is longitudinal or transverse with respect to the centromeric fibers. The other three types of quartets (A, D, and E) are no more useful in deciding this question, since they could all result from aberrant centromere behavior at either MI exclusively, MII exclusively, or at both divisions in succession. It seems probable, however, that aberrant behavior is confined to MI for several reasons: there is definite evidence that certain aberrant types do occur at this stage (types II and III); there is good though not conclusive evidence that other types also occur (that is, types Ib and Ic); all five quartet types recorded at AII may be explained as a result of the postulated centromere behavior in these certain or probable types at MI alone without invoking misdivision at MII; and finally, there is no cytological evidence for misdivision at MII or AII, though such evidence might be rather difficult to obtain.

Hypotheses other than the one presented may be suggested to account for the observed anomalous centromere behavior. DARLINGTON (1939), on the basis of an analysis of the behavior of univalents at meiosis, has suggested that centromeres have a dual structure consisting of internal centrogenes connecting chromatid arms and an enclosed fluid element which is the essential organ of division. The direction of division of the fluid element is presumably determined by the orientation of the centrogenes. He further maintains that the centrogenes divide after the true genes and that "misdivision is due to their exceptionally failing to divide in time for the explosion of the centric fluid which is precocious in univalents at meiosis." A modified form of this hypothesis could explain the behavior of the SML tetrad. However, it is not very clear why the failure of centrogenic division should necessarily lead to a transverse or oblique rather than to a longitudinal division of the centromere at MI. Another possible type of behavior involves the fusion of the centromeres of the two SML chromosomes at prophase of meiosis, thus producing a single centromere with eight attached arms similar to a diplochromosome at mitosis. The subsequent division of the giant single centromere at MI would then have to occur either transversely, longitudinally, or in part in both planes, but always so as to separate the eight arms in a quantitatively symmetrical (4 by 4) fashion. There is no evidence for such a centromeric fusion,

however, and the hypothesis initially suggested appears to be simpler and more probable.

It has been indicated that there are certain definite relations among the various types at AI and AII; thus, type II can give rise only to type B; type III only to type C; type I (as a group) only to types A, D, and E. Consequently the percentages of these related types at AI and AII should correspond. The figures given in tables 2 and 3 indicate that there is a fair, though not exact correspondence. Types III (9.4 percent) and C (7.1 percent) agree very well; type I (48.8 percent), however, is the most frequent type at AI, whereas its corresponding types at MII (A, D, and E) show a combined frequency (39.7 percent) less than that of type B (53.2 percent). These discrepancies are not large, however, and may be due to inadequate size of the sample or to the combination of non-comparable data, since the observations are from slides made on several different days.

It may be recalled, in conclusion, that the *mitotic* behavior of the centromeres of SML chromosomes in somatic cells is entirely normal. Furthermore the centromeres of newly formed iso-chromosomes apparently divide and disjoin normally at the microspore mitosis. Only at meiosis is evidence of aberrant behavior found. In this respect the present results are similar to those obtained by RHOADES and VILKOMERSON (1942) who describe a peculiar type of anaphase chromosome separation which occurs only at meiosis. Such investigations of atypical chromosome behavior provide further evidence of the ways in which mitosis and meiosis differ and also reveal significant information concerning the structure and function of chromosomes and their parts.

SUMMARY

A unique type of centromere behavior at meiosis has been found in a single plant of Gasteria. This plant possesses a diploid chromosome complement corresponding in morphology and number (14) to that characteristic of the genus. No evidence has been found of aberrant centromere behavior at mitosis. At first metaphase of meiosis, however, the centromeres of one of the seven tetrads behave in an anomalous fashion in a considerable fraction of the pollen mother cells. As a result two complementary types of iso-chromosomes—one having two identical long arms, the other two identical short arms—in addition to the usual chromosome type, arise directly from this tetrad. The other six tetrads go through both meiotic divisions in an entirely regular manner. Consequently, three types of microspores are formed at the end of meiosis, all having the same chromosome number (7) but differing in the kind of chromosome received from the division of the aberrant tetrad. About half the microspores receive a normal chromosome from this tetrad; the other half receive iso-chromosomes, the two complementary types being equally frequent. Those microspores having the morphologically normal chromosome possess a complete haploid complement and develop normally; those carrying either of the two iso-chromosome types are homozygous deficient for different parts of the haploid complement. One of these microspore

classes (the one containing the larger cytological deficiency) fails to develop; the development of microspores in the other class is delayed, but such cells eventually pass through the first post-meiotic mitosis, at which time the iso-chromosomes divide and separate normally.

Observations at meiosis reveal that the aberrant centromere behavior is probably confined to the first division. The evidence indicates that at MI the two centromeres of the affected tetrad divide precociously, either transversely or longitudinally. The various possible combinations of transverse and longitudinal division of the two centromeres are followed by the union by twos of half-centromeres, either sister or non-sister halves uniting. These unions occur in all possible ways. Consequently anaphase separation at AI is always quantitatively symmetrical (one centromere and four arms going to each pole), but may be qualitatively asymmetrical (different combinations of arms may go to opposite poles). The dyads behave normally on the spindle at AI and give rise to five distinct types of quartets at AII with respect to chromosome morphology and arrangement. An interpretation of the aberrant meiotic behavior in terms of a suggested internal structure of the tetrad centromeres is presented and discussed.

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INHERITANCE IN NICOTIANA TABACUM. XVI. STRUCTURAL DIFFERENCES AMONG THE CHROMOSOMES OF A SELECTED GROUP OF VARIETIES

GAAFAR S. MALLAH

Division of Genetics, University of California, Berkeley, California

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THE studies reported herewith deal with the structural relations among the chromosomes of fifteen horticultural varieties of *Nicotiana Tabacum*. They were initiated as a consequence of the discovery that hybrids of the standard variety, Purpurea, with the variety Cuba exhibit 19II+⊙6+⊙4 instead of 24II chromosomes in meiosis. For the purposes of the study 14 varieties of *N. Tabacum* were first crossed with Purpurea; then those varieties which produced hybrids exhibiting evidence of structural difference were intercrossed in order to determine their structural affinities.

THE VARIETIES STUDIED

The 15 varieties employed in the study include a number of commercial types, a few possessing unusual or freak characters, and some local varieties collected by DR. T. H. GOODSPEED in South America. The names, aside from those of established commercial varieties, are mainly locality or other designations employed for convenience. For purposes of precise identification, the UCBG (UNIVERSITY OF CALIFORNIA BOTANICAL GARDEN) number is also given. These varieties have all been continued by selfing, and they exhibit 24II chromosomes in meiotic metaphases, as revealed by acetocarmine smears of microsporocytes. The list follows:

1. Purpurea (UCBG 06-25), originally from the MISSOURI BOTANICAL GARDEN. Described by SETCHELL (1912) and since employed as the standard variety of reference for our investigations of *N. Tabacum*.

2. Consolation (UCBG 40-22), a variety with yellowish-green leaf color from Puerto Rico (NOLLA 1934).

3. Trelease Turkish (UCBG 40-24), a Turkish-type variety from PROFESSOR SAM F. TRELEASE, COLUMBIA UNIVERSITY.

4. Belge (UCBG 40–25), an old Canadian variety received from DR. N. A. MACRAE.

5. Station Standup (UCBG 40-26), a modern Canadian burley variety (MACRAE and HASLAM 1935).

6. Holmes Samsoun (UCBG 40-28), a Turkish variety incorporating the mosaic resistance of *N. glutinosa* (HOLMES 1938).

7. Cuba (UCBG 14-200), a white-flowering parthenocarpic variety (GOODSPEED 1915) originally received from MRS. ROSE HAIG THOMAS.

8. Ambalema (UCBG 37-51), a mosaic-resistant variety collected by NOLLA in Colombia (NOLLA and ROQUE 1933).

9. Huadquina (UCBG 36-170), a distinctive local type collected by DR. T. H. GOODSPEED in the vicinity of Machu Picchu, Peru.

10. Serrate (UCBG 40-23), a variety with somewhat incised leaves distributed by the U. S. DEPARTMENT OF AGRICULTURE.

11. Ceniza (UCBG 40-27), a variety with hairy filaments and glaucous leaves from Puerto Rico (NOLLA 1934).

12. Bergerac Catacorolla (UCBG 40-29), a freak variety, flowers with supernumerary reversed corolla attached externally to the tube at the base of the infundibulum (ARISZ 1927), collected by DR. H. P. OLMO at Bergerac, France.

13. Maryland Mammoth (UCBG 31-1), a commercial mammoth variety supplied by E. G. BEINHART.

14. Apolo-I (UCBG 39-245), a very distinctive weed-like local type collected by T. H. GOODSPEED in Bolivia.

15. Apolo-II (UCBG 39-246), a commercial-type variety collected by T. H. GOODSPEED at the same locality as the preceding variety.

STRUCTURAL DIFFERENCES AMONG THE VARIETIES

The hybrids of the 14 varieties with the standard type, Purpurea, may be classified in three groups with respect to MI associations:

1. Those with 24 bivalents (fig. 1), indicating structural identity with Purpurea. Nine varieties appear to be structurally identical with Purpurea—namely, Consolation, Trelease Turkish, Belge, Station Standup, Ambalema, Serrate, Bergerac Catacorolla, Maryland Mammoth, and Apolo-II; but a few, for example Belge and Apolo-II, gave some evidence of intrachromosomal dislocations relative to Purpurea.

2. A hybrid with 23 bivalents and two univalents (fig. 3). The presence of a non-conjunctional pair of chromosomes was a unique feature of the Purpurea-Holmes Samsoun hybrid.

3. Those with multivalent (amphibivalent) associations. The hybrids of this class arise from the varieties: Apolo-I, Ceniza, Huadquina, and Cuba. The number and character of the associations is recorded in the first line of table 1.

In order to explore the problem further, the five varieties which gave atypical results with Purpurea were intercrossed. These results are also recorded in table 1, where the number and character of associations only are indicated, the remaining chromosomes regularly forming bivalents.

CHARACTERISTIC FEATURES OF THE COMPLEXES

Not only does the structural analysis indicate the distinctness of the complexes, but their variation in association testifies to the same conclusion. The parental varieties, as selfed lines, consistently exhibit 24 bivalents in meiosis. Most of the bivalents are attached at both ends, but rods are sometimes seen as a consequence of chiasma formation in one arm only. The chiasma frequency is comparatively low; instances of two chiasmata in one arm are rather infrequent. The behavior of the different complexes is noted briefly below:

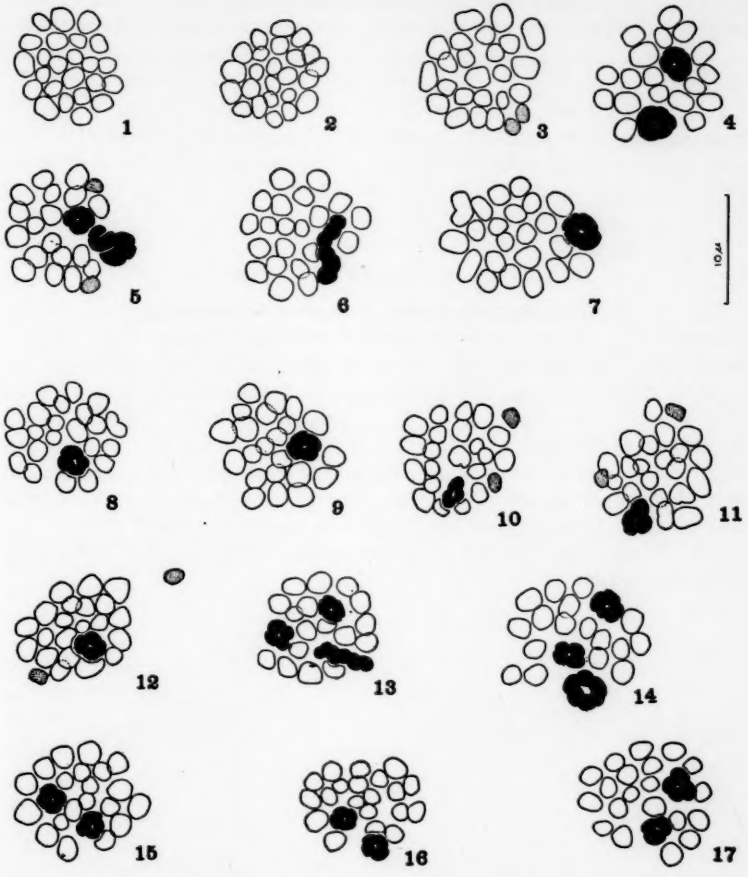

Fig. 1.—Purpurea-Serrate, 24II.—Fig. 2. Holmes Samsoun, 24II.—Fig. 3. Purpurea-Holmes Samsoun, 23II+2I.—Fig. 4. Purpurea-Cuba, 19II+1VI+1IV.—Fig. 5. Holmes Samsoun-Cuba, 18II+1VI+1IV+2I.—Fig. 6. Apolo-I-Ceniza, 21II+1VI.—Fig. 7. Purpurea-Huadquina, 22II+1IV.—Fig. 8. Purpurea-Ceniza, 22II+1IV.—Fig. 9. Purpurea-Apolo-I, 22II +1IV.—Fig. 10. Holmes Samsoun-Huadquina, 21II+1IV+2I.—Fig. 11. Holmes Samsoun-Ceniza, 21II+1IV+2I.—Fig. 12. Holmes Samsoun-Apolo-I, 21II+1IV+2I.—Fig. 13. Cuba-Ceniza, 17II+1VI+2IV.—Fig. 14. Cuba-Apolo-I, 17II+1VI+2IV.—Fig. 15. Apolo-I-Huadquina, 20II+2IV.—Fig. 16. Ceniza-Huadquina, 20II+2IV.—Fig. 17. Cuba-Huadquina, 20II +2IV.

The figures are from camera-lucida drawings of microsporocytes at MI. Bivalents, outlined; univalents, stippled; multivalent associations, solid black.

TABLE 1

Maximum associations observed in various Tabacum hybrids.

	APOLO-I	CENIZA	HUADQUINA	CUBA	HOLMES SAMSOUN
Purpurea	4	4	4	6+4	1+1
Holmes Samsoun	4+1+1	4+1+1	4+1+1	6+4+1+1	
Cuba	6+4+4	6+4+4	4+4		
Huadquina	4+4	4+4			
Ceniza	6				

The non-conjunctional complex

This complex was observed in every cross of Holmes Samsoun with the other varieties (fig. 2, 3, 5, 10–12). The two univalents appear to be approximately the same size, and they are always located on the periphery of the plate, as is normally the case for unassociated chromosomes (OLMO 1936). They appear to be entirely independent. Sometimes they are located on opposite sides of the plate; sometimes close together, in which case, however, they usually lie in different planes. No case of association has ever been observed between them, nor have they ever been seen to conjugate with other chromosomes. A single chromatin bridge was observed in a cell of the Cuba-Holmes Samsoun hybrid, but it was impossible to connect it with the non-conjunctional pair.

Six-chromosome complexes

There are two different six-chromosome complexes; (a) the one appearing in hybrids of Cuba with Purpurea, Holmes Samsoun, Ceniza, and Apolo-I (fig. 4, 5, 13, 14) and (b) that of the Ceniza-Apolo-I hybrid (fig. 6). The former rarely forms a complete circle; often it appears as a chain of six, but more frequently it is broken into smaller units. Presumably these breaks are a consequence of failure of chiasma formation, dependent upon the length of the associated segments (DARLINGTON 1929), and do not occur at random. The number of breaks observed varied from one to five. By no means, however, were all the possible configurations observed; a very large number of plates would have been necessary to have made such a demonstration possible. In one instance (fig. 5) a complete ring appeared to be half twisted, resembling a figure-of-eight, but the occurrence of this configuration must remain doubtful in view of the high degree of contraction of the chromosomes. The latter complex very rarely produces a complete ring or chain of six chromosomes; but in the majority of instances a chain of four chromosomes and a bivalent are seen instead. Both complexes take up a peripheral position in the plate.

Four-chromosome complexes

The four-chromosome complexes (fig. 7–12) are best observed in the hybrids of Purpurea and Holmes Samsoun with Cuba, Huadquina, Ceniza and

Apolo-I, where only a single such complex is present. When these complexes form complete circles, they seem to have no preference for any definite position in the metaphase plate. As the structural analysis shows, the complexes of the four hybrids of Purpurea involve different chromosomes; and since Holmes Samsoun is structurally identical with Purpurea, except for the chromosome of the non-conjunctional pair, its hybrids with these four varieties form a parallel series. Correspondingly the frequencies of specific types of associations differ in the different hybrids, but they appear to be identical in the equivalent pairs of hybrids involving Purpurea and Holmes Samsoun. The configurations observed and their approximate frequencies for a group of selected hybrids are recorded in table 2. For convenience we may call these complexes the

TABLE 2

Types and frequencies of associations of the single four-chromosome complexes in Tabacum hybrids.

TABACUM HYBRIDS	CIRCLE OF FOUR	CHAIN OF FOUR	CHAIN OF THREE ONE SINGLE	TWO PAIRS	ONE PAIR TWO SINGLES	TOTALS
Huadquina-Purpurea	33	8	—	9	—	50
Ceniza-Purpurea	1	2	2	30	1?	36
Ceniza-Holmes Samsoun	2	1	1	30	—	34
Apolo-I-Purpurea	37	1	—	2	—	40
Apolo-I-Holmes Samsoun	18	1	—	1	—	20
Cuba-Purpurea	21	1	—	3	—	25

Apolo-I, the Ceniza, the Huadquina, and the Cuba complexes. The Apolo-I and Cuba complexes form a complete circle in the majority of the cells, indicating that failure of chiasma formation in one or more of the four arms of the association is very rare. The Huadquina complex likewise frequently forms a complete circle, but in about twenty percent of cases it forms an open chain and in an additional twenty percent a pair of bivalents. The Ceniza complex, on the other hand, rarely forms either a complete circle or a chain quadrivalent, but usually forms two bivalents. Presumably these differences depend upon the lengths of the translocated segments.

In the majority of cells, as seen in polar view, when the four-chromosome complexes form a complete circle they appear as flat rings in the plate; only rarely, in about five percent of the cells, are they arranged vertically—that is, non-disjunctionally. Whether the four chromosomes are really in one plane or in a zigzag arrangement usually cannot be determined; but occasionally it is possible by careful focussing to observe that adjacent chromosomes lie at different levels.

Two four-chromosome complexes are present in the Cuba hybrids with Ceniza, Apolo-I, and Huadquina (fig. 13, 14, 17) and in the Huadquina hybrids with Apolo-I and Ceniza (fig. 15, 16). As shown by the structural analysis, these complexes are the same as those described above, except for one of the two in the Cuba-Huadquina hybrid; one is the Cuba complex, which, as

described above, usually forms a complete circle; the other is from the Cuba-Purpurea six-chromosome complex by reason of the fact that Cuba and Huadquina have one relatively translocated chromosome (8–10) in common. It frequently breaks down and appears to consist of large chromosomes. When two four-chromosome complexes are present in a cell, they appear to behave independently and to exhibit the same types and frequencies of configurations as have been described individually above.

DISCUSSION

Considering the observations recorded in table 1, the chromosomal structure of the varieties may be formally analyzed relative to that of Purpurea as depicted in table 3. The results show that in a relatively small sample of *Tabacum*

TABLE 3

Structural analysis of the Tabacum varieties.

Purpurea	1–2	3–4	5–6	7–8	9–10	11–T–12	13–14	15–16	17–18	19–20 . . .
Holmes Samsoun	1–2	3–4	5–6	7–8	9–10	11–G–12	13–14	15–16	17–18	19–20 . . .
Cuba	1–3	2–4	5–7	6–9	8–10	11–T–12	13–14	15–16	17–18	19–20 . . .
Huadquina	1–2	3–4	5–6	7–9	8–10	11–T–12	13–14	15–16	17–18	19–20 . . .
Ceniza	1–2	3–4	5–6	7–8	9–10	11–T–12	13–15	14–16	17–18	19–20 . . .
Apolo-I	1–2	3–4	5–6	7–8	9–10	11–T–12	13–14	15–17	16–18	19–20 . . .

varieties structural changes arising from reciprocal translocation have occurred in no less than eight chromosomes. These structural alterations in the chromosomes appear to be confined mainly to local varieties collected in South America; the standard commercial varieties, and derivatives from them, all appear to be identical structurally with Purpurea, the variety chosen arbitrarily as the point of reference for our *Tabacum* studies. Two of the varieties, Cuba and Huadquina, have a relatively translocated chromosome in common, which may bespeak a common source of origin. Huadquina is a recent accession collected near Machu Picchu, Peru, but no record of the New World source of origin of the other appears to be available, except that it may have come from Cuba or Tobago. In view of the large number of translocations revealed by this preliminary study, further investigations are in progress involving a number of other varieties collected by T. H. GOODSPEED in various South American localities.

Of special interest is the situation in the mosaic-resistant variety, Holmes Samsoun, one chromosome of which fails to conjugate with the corresponding member of the standard *Tabacum* set. This variety was deliberately established in order to incorporate in *Tabacum* the well-known necrotic type of response of *N. glutinosa* to infection with the mosaic virus. The normal F_1 *glutinosa-Tabacum* hybrid exhibits the necrotic type of response, but it is completely sterile. To overcome the sterility, HOLMES (1938) resorted to the use of the amphidiploid *glutinosa-Tabacum* (CLAUSEN and GOODSPEED 1925), which gives a fertile sesquidiploid hybrid with *Tabacum*. In the origin of Holmes Samsoun, the Turkish variety, Samsoun, was first crossed with amphidiploid

glutinosa-Tabacum, then the sesquidiploid hybrid thus produced was back-crossed to Samsoun, and thereafter successive selfed generations, selected for mosaic resistance, were raised until eventually a constant mosaic-resistant type was established, the Holmes Samsoun of this paper. Our supply of seed of the variety was kindly provided by DR. F. O. HOLMES himself. It is not clear just now how the mosaic resistance became incorporated in the variety, since no parallel cytological studies were conducted, but the results reported herewith seem to indicate that an entire *Tabacum* chromosome has been displaced by a corresponding *glutinosa* chromosome, which although apparently functionally a satisfactory substitute is nevertheless unable to conjugate with its *Tabacum* analogue. In the succeeding paper in this series, GERSTEL (1943) reports a demonstration of the connection between this non-conjunctional pair of chromosomes and the mosaic-resistant feature.

SUMMARY

The structural relations of the chromosomes of 15 horticultural varieties of *N. Tabacum* were studied.

Ten of them including the standard variety, Purpurea, appear to be identical structurally. All of the strictly commercial-type varieties of the sample were included in this group.

One variety, Holmes Samsoun, gives hybrids which exhibit a non-conjunctional pair of chromosomes, apparently on account of replacement of a *Tabacum* by an entire *glutinosa* chromosome in the genesis of this mosaic-resistant variety.

Three varieties—Apolo-I, Ceniza, and Huadquina—contain single reciprocal translocations relative to the standard type.

One variety, Cuba, has three reciprocal translocations; one between two and two among three chromosomes.

Structural analysis of the translocation types demonstrate that they involve eight different chromosomes.

The varieties Cuba and Huadquina have a relatively translocated chromosome in common.

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I am deeply indebted to PROFESSOR R. E. CLAUSEN for suggesting and supervising this work. I wish to thank DR. D. R. CAMERON and the staff of the Division of Genetics for the help and facilities they offered me.

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INHERITANCE IN NICOTIANA TABACUM. XVII. CYTOGENETICAL ANALYSIS OF GLUTINOSA-TYPE RESISTANCE TO MOSAIC DISEASE

D. U. GERSTEL

Division of Genetics, University of California, Berkeley, California

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MOST varieties of *Nicotiana Tabacum* are susceptible to tobacco mosaic. Upon infection the virus spreads in systemic fashion throughout the plant; leaves and flowers exhibit chlorotic streaking; quality and yield are severely reduced. *N. glutinosa*, however, exhibits resistance to the disease, owing to the fact that the virus becomes isolated in necrotic spots. Transfer of this resistance to *N. Tabacum* is not easily effected, because the F_1 *glutinosa-Tabacum* hybrid is sterile. But HOLMES (1938) was able to make the transfer by employing the amphidiploid *glutinosa-Tabacum* hybrid (CLAUSEN and GOODSPEED 1925) which exhibits the *glutinosa* type of response to the virus. By crossing amphidiploid *glutinosa-Tabacum* to the Turkish *Tabacum* variety Samsoun, a fertile hybrid was secured which through subsequent backcrossing followed by selfing and selection for resistance led to the establishment of a pure breeding variety of the Samsoun type resistant to mosaic. For purposes of identification this variety is called Holmes Samsoun.

This accomplishment raised some interesting cytological problems, for *glutinosa* chromosomes do not conjugate regularly with *Tabacum* chromosomes (GOODSPEED 1934), and the mode of incorporation of the resistance in *Tabacum* was not, therefore, clear. Through the courtesy of DR. HOLMES, we have had the opportunity of studying the problem from this point of view. In the preceding paper in this series, MALLAH (1943) has shown that while Holmes Samsoun itself, like other varieties of *N. Tabacum*, exhibits 24 pairs of chromosomes in the meiotic metaphase, the F_1 hybrids of Holmes Samsoun with other *Tabacum* varieties regularly exhibit an unassociated, non-conjunctional pair of chromosomes. Twenty-five metaphase plates in each of five hybrids failed to exhibit any deviation from this behavior. It seemed probable, therefore, that incorporation of resistance to mosaic in Holmes Samsoun had been achieved by substitution of the entire *glutinosa* chromosome, which carried the resistance factor, for a *Tabacum* chromosome. The investigations reported herewith are concerned with a determination of the relation between the non-conjunctional pair of chromosomes and the transmission of mosaic resistance.

METHODS AND RESULTS

For the purpose of testing the chromosomal relations, reciprocal backcross progenies of F_1 Purpurea-Holmes Samsoun×Purpurea were grown. The F_1 plants exhibited 23 bivalents and two univalents. They were necrotic in their reaction to the mosaic virus and had a relatively high pollen abortion. In order

to avoid effects of selection consequent upon differences in vigor of different seedling types, seeds were germinated on moistened filter paper, and every seedling that germinated from a counted number of seeds was grown.

From the backcross, F_1 Purpurea-Holmes Samsoun ♀ × Purpurea ♂, 112 plants passed the seedling stage. These plants were tested individually for mosaic reaction by brushing detached leaves with a "stock solution" of the virus, after which they were incubated in a moist chamber. This procedure represented a modification of the method devised by DUNDAS (1934) for studying mildew resistance in beans. The leaves either responded with the production of conspicuous necrotic lesions, or they exhibited no signs at all (non-necrotic type). It was assumed that these latter were actually susceptible, since a positive chlorotic response cannot be obtained on detached leaves. A few non-necrotic plants were inoculated. They developed typical chlorotic symptoms. Pollen abortion studies disclosed that the plants were divisible roughly into two categories—a low abortion group (range 44–95 percent of good pollen) exhibiting pollen approximately equivalent in this respect to the parental types and a high abortion group (range 8–17 percent of good pollen) approximately equivalent to F_1. The results were as follows:

MEIOTIC METAPHASE	CHROMOSOMAL TYPE	MOSAIC RESPONSE	POLLEN ABORTION	NO. OF PLANTS	PERCENTAGE	EXPECTED PERCENTAGE
23II+2I	TG	necrotic	high	19	17.0	14.3
24II+1I	TTG	necrotic	low	2	1.8	4.5
23II+1I	T	non-necrotic	high	66	58.9	61.6
24II	TT	non-necrotic	low	25	22.3	19.6
				112	100.0	100.0

In the reciprocal backcross, Purpurea ♀ × F_1 Purpurea-Holmes Samsoun ♂, only two classes appeared—namely:

MEIOTIC METAPHASE	CHROMOSOMAL TYPE	MOSAIC RESPONSE	POLLEN ABORTION	NO. OF PLANTS	PERCENTAGE
23II+2I	TG	necrotic	high	20	46.5
24II	TT	non-necrotic	low	23	53.5

These results are readily explainable on the assumption that the non-conjunctional pair of chromosomes consists of one *Tabacum* (T) and one *glutinosa* (G) chromosome. The presence of the latter determines necrotic mosaic response. Under this assumption the parents are Purpurea = TT, Holmes Samsoun = GG, and F_1 = TG. In view of the non-conjunction of TG four classes of gametes are produced: TG—T—G—O, which yield upon backcrossing to Purpurea four classes of zygotes: TTG—TT—TG—T, which are identified with the four classes of zygotes based upon mosaic response and pollen abortion.

The numerical proportion of the classes is roughly in accord with expectation. Monosomic studies (CLAUSEN and GOODSPEED 1926) have shown that

an unassociated chromosome is included in 20 to 25 percent of the gametes, but the proportion of functional gametes is frequently subject to marked secondary modification. In the above results T was transmitted to 24.1 percent of the offspring and G to 18.8 percent, indicating that the female ratio of transmission was not greatly modified by secondary phenomena, such as for example selective ovule abortion.

The expected results, set up on the basis of independent transmission of the two chromosomes in the ratios noted, are substantially in agreement with the actual results. They exhibit only a slight bias in favor of balanced (TT and TG) as against unbalanced monosomic (T) and trisomic (TTG) classes.

Monosomic and trisomic studies also have revealed that 23- and 25-chromosome male gametes rarely reach the ovules in competition with normal 24-chromosome gametes. Consequently only the T and G classes of gametes are effective in the reciprocal cross and only two classes of zygotes appear in approximately equal numbers.

Zygotes containing T are somewhat in excess of those containing G in both crosses, but the numbers are not large enough to establish significance for this bias.

DISCUSSION

It is remarkable that the substitution of a chromosome from another species for a *Tabacum* chromosome does not result in any functional disturbance. The Holmes Samsoun variety seems to be of normal vigor and to have full reproductive capacity. It would be natural to assume that the two chromosomes are closely homologous. But no pairing between them has been observed in any instance contrary to the usual behavior of homologous chromosomes. On the other hand, it may be supposed that the hereditary substance contained in the *Tabacum* chromosome in question is not necessary for the plant. But as yet no viable nullosomic types have been found as derivatives from monosomics in tobacco, and male gametes with 23 chromosomes are rarely viable. However, the *glutinosa* chromosome is able to replace the *Tabacum* chromosome in zygotes as well as in gametes. It must be concluded that the *glutinosa* chromosome contains the essential factors present in the replaced *Tabacum* chromosome. Their failure to pair, however, would seem plausible if structural changes could be shown to have taken place in the phylogeny of the species, involving the chromosome in question.

The difference between the new resistant variety and the chlorotic varieties consists in an entire chromosome, not merely in one or several genetic factors. Pollen and ovule transmission, therefore, are not the same, and reciprocal crosses with Holmes Samsoun hybrids give divergent results. The percentage of resistant plants in the offspring is about doubled when the pollen parent is the carrier of the *glutinosa* chromosome with the resistance factor, for in this case gametes with 23 chromosomes are eliminated. Furthermore, the probability of obtaining TTG plants from a TG pollen parent is extremely low, and this undesirable type will not, therefore, occur in the selected necrotic offspring. The use of heterozygous plants as pollen parents thus will provide more and better material for selection than the reciprocal cross.

SUMMARY

In the mosaic resistant *Tabacum* variety, Holmes Samsoun, a pair of *glutinosa* chromosomes has been substituted for a pair of *Tabacum* chromosomes.

In hybrids of Holmes Samsoun with Purpurea the *glutinosa* chromosome fails to conjugate with its *Tabacum* analogue, although it appears to be physiologically equivalent to it.

The transmission of mosaic resistance parallels distribution of the non-conjunctional pair of chromosomes, demonstrating that the factor or factor complex for mosaic resistance is borne in one of these chromosomes.

In heterozygous plants, as a consequence of non-conjunction, about twenty percent of female gametes contain the T and about an equal proportion the G-chromosome; the remaining sixty percent consists mainly of 23-chromosome gametes and a few with 25 chromosomes. All are functional.

On the male side unbalanced 23- and 25-chromosome gametes very rarely function, so that a 1:1 transmission ratio is secured on backcrossing heterozygous plants.

The plant breeder will find it advantageous to use heterozygous plants as pollen parents in transferring mosaic resistance to other *Tabacum* varieties.

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CORRIGENDA

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Page 568, line 24, for "B_1 to P_2 (dominant parent)" read "B_1 to P_2 (recessive parent)."

Page 571, line 7, for "the number gene by pairs by which " read "the number of gene pairs by which."

Page 572, line 1, read

$$\frac{2^n (\log KK + \log Kk)}{2\,(2^n)} + (\log V_1 + V_2 + \cdots + \log V_n).$$

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Page 14, line 3, for "A 2 indicates" read "A ② indicates."

Page 24, line 15, for "a simple gene" read "a single gene."

Page 59, line 23, for "discussion of p. 59" read "discussion of p. 57."

Page 301, line 20, for "teminology" read "terminology."

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